12-2008

Synthesis of Marine Chemicals and Derivatives as Potential Anti-Cancer Drugs.

Laude Bannerman-Akwei
East Tennessee State University

Synthesis of Marine Chemicals and Derivatives as Potential Anti-Cancer Drugs

A thesis

presented to

the faculty of the Department of Chemistry

East Tennessee State University

In partial fulfillment

of the requirements for the degree

Master of Science in Chemistry

by

Laude Bannerman-Akwei

December 2008

Dr. Yu Lin Jiang, Ph.D Chair

Dr. Ismail Kady, Ph.D

Dr. David Young, Ph.D

Keywords: Cancer, Drugs, Marine, Natural Products, Synthesis

ABSTRACT

Synthesis of Marine Chemicals and Derivatives as Potential Anti-Cancer Drugs

by

Laude Bannerman-Akwei

Two natural marine compounds, 3-bromo-4,5-dihydroxybenzaldehyde **2** and 2,3-dibromo-4,5-dihydroxybenzaldehyde **5** together with two novel derivatives, 3-bromo-5-(*tert*-butyl-dimethyl-silanyloxy)-4-hydroxybenzaldehyde **3** and 1-bromo-2,3-dimethoxy-5-nitrooxy-methylbenzene **9**, were synthesized. Compounds **2**, **3**, and **5** were evaluated for their biological activity towards the inhibition of prostate cancer cell growth using staurosporine as a positive control. All three compounds have shown significant inhibition of prostate cancer cell growth. Compound **9** is yet to be evaluated.

DEDICATION

This thesis is dedicated to my family and Dr. Clement Adokwei Akwei of blessed memory.

ACKNOWLEDGMENTS

I am grateful to the Lord Almighty for a successful end to this thesis. Indeed his Grace has been sufficient for me and he has seen me through it all.

I also want to express my sincere gratitude to Dr Yu-Lin Jiang for giving me the opportunity to work on this exhilarating project, his academic guidance, encouragement, and patience throughout the course of this project. Special thanks to Dr. Ismail Kady and Dr. David Young for their advice and patience while serving on my thesis committee. My appreciation also goes Dr. Stone and his workers at the school of medical sciences ETSU for the biological activity analysis.

I am grateful to ETSU for granting me the opportunity to pursue my graduate studies here and the faculty and staff of the department of chemistry for all their support and the knowledge they have imparted in me.

Finally, I want to thank my friends for their support and everyone who has contributed to my success here at ETSU.

CONTENTS

LIST OF SCHEMES

LIST OF ABBREVIATIONS

Ac	acetyl
DMF	dimethylformamide
DMSO	dimethylsulfoxide
Et	ethyl
Hrs	hours
GCMS	gas chromatography mass spectrometer
IR	infrared
Lit	literature
mp	melting point
Me	methyl
NMR	nuclear magnetic resonance
rt	room temperature
TBS	*tert*-butyldimethylsilyl
THF	tetrahydrofuran
S_N2	bimolecular nuclophilic substitution

CHAPTER 1

INTRODUCTION

The need to discover new drugs has become imperative due to the advent of many drug resistant diseases. Nature has continuously provided human beings with a broad and structurally diverse arsenal of pharmacologically active compounds that continue to be used as highly effective drugs to combat a multitude of deadly diseases or as lead structures for the development of novel synthetically derived drugs that mirror their models from nature [1]. Natural product chemists therefore continue to explore nature for new sources of lead compounds in search of new drugs and medicines as well as improving the efficiency of drugs [2]. Drugs of natural origin have been categorized into three groups; the original natural product, semi-synthetic products derived form the chemical modifications of natural products and total synthetic analogues based on natural product models or pharmacophores [3].

Most of the 877 new small molecule chemical entities introduced as drugs worldwide during 1981-2002 were either natural products, natural product derivatives, or natural product mimics. The percentages were even greater when considering only the antibacterial (79%) and anticancer (74%) compounds [4]. Despite the continuous research being done by natural product chemists, the rate of discovery of truly novel natural product drugs has actually decreased. Reasons for this fact are related to high cost and time consuming of conventional programs in natural products, which led to the exploitation of modern high-throughput screening and combinatorial strategies by the pharmaceutical industry to generate new lead structures. However, far from being competitive, combinatorial and natural product chemistry

should complement on a synergistic perspective, because nature continues to be the most diverse and active compound library known [5].

More than 70% of our planet's surface is covered by sea and these waters harbor approximately 75% of all living organisms. The marine environment has therefore become an invaluable resource for new drug discoveries due to its diversity of life and associated secondary metabolites. Many species of marine organisms have evolved and developed the enzymatic capability to produce chemical entities that might serve not only as defense mechanism systems against micro and macro predators but also as regulators of biological function. These compounds offer a good starting point in the quest for innovative marine derived anticancer drugs. In recent years, marine natural product bioprospecting has yielded a considerable number of drug candidates and most of these molecules are still in preclinical or early clinical development [6, 7, 8].

Marine Natural Products

The Discovery Phase

The pioneering work of Bergman et al. (1951) in the discovery of the biologically active, pharmaceutically important, and novel arabino-nucleosides from the sponge *Cryptotethya crypta* sparked the interest in marine natural product and served to highlight the biomedical potential of the field [9]. Although the initial work of Bergman et al. was curiosity driven, the discovery of the arabino-nucleosides now serves as lead structures for the development of antiviral drugs such as ara-A and the anticancer drug for leukemia ara-C [1].

Current Perspective

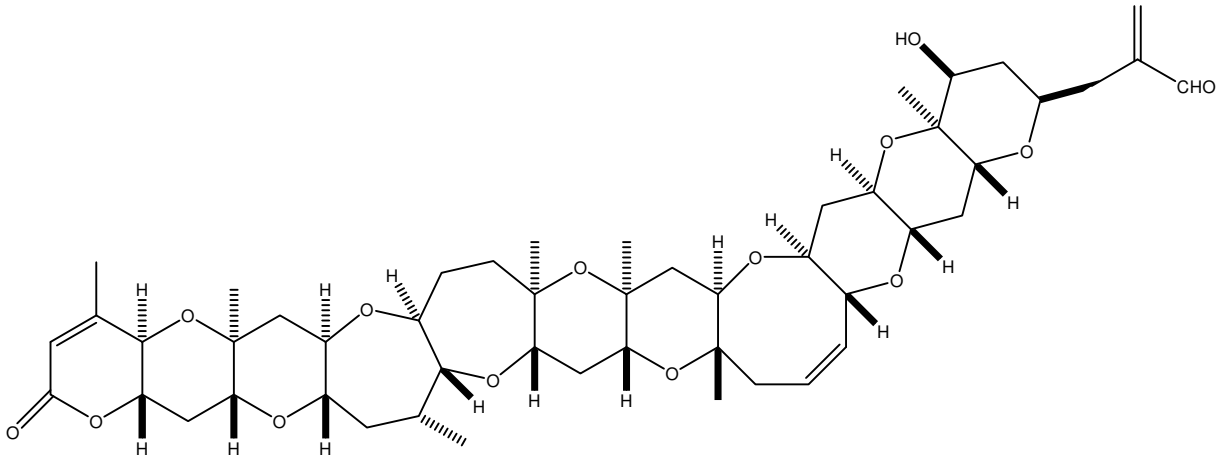
The field has blossomed and matured since the pioneering work of Bergman et al. with the isolation of several different compounds. Most of these compounds have shown significant cytotoxic activity (mostly anticancer or antitumor agents) than with terrestrial sources. Thus, whereas an estimated 1 in 10,000 compounds of terrestrial origin screened for antitumor activity yield a candidate for drug development, the corresponding figure for marine sources is closer to 1 in 100 [6]. A good number of promising compounds that have been identified are either already at advance stages of clinical trials or have been selected as promising candidates for extended preclinical evaluation [1].

In 2006, 779 new compounds together with their relevant biological activities, source organisms, and country of origin were published in 283 articles [10]. With over 200,000 invertebrate and algal species in the ocean [11] the future of marine natural product chemistry can only be bright.

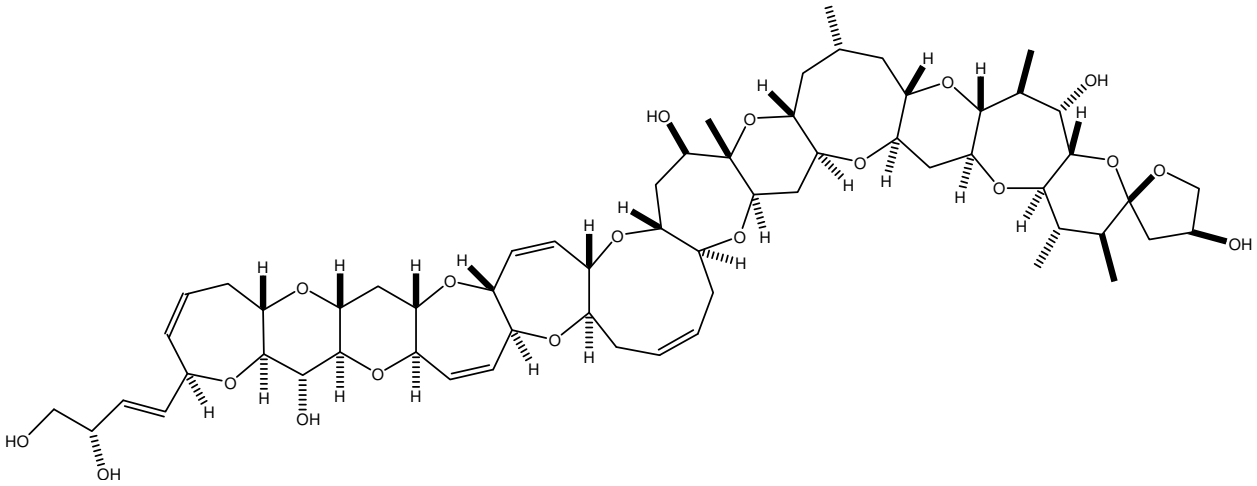
The Chemistry of Marine Natural Products

Marine Toxins

This is dominated by the polyether toxins and it includes; brevetoxin B **1** isolated from the dinoflagellate *Gymmodinium breve*[12], ciguatoxin **2** extracted from moray eels *Gymmothorax javanicus* [13], maitotoxin **3** isolated from *Gambierdiscus toxicus*[14, 15], and yessotoxin **4** isolated from scallops *Patinopecten yessoensis* implicated in diarrheic shellfish poisoning and from the same source pectenotoxin-1 **5** has also been isolated [16].

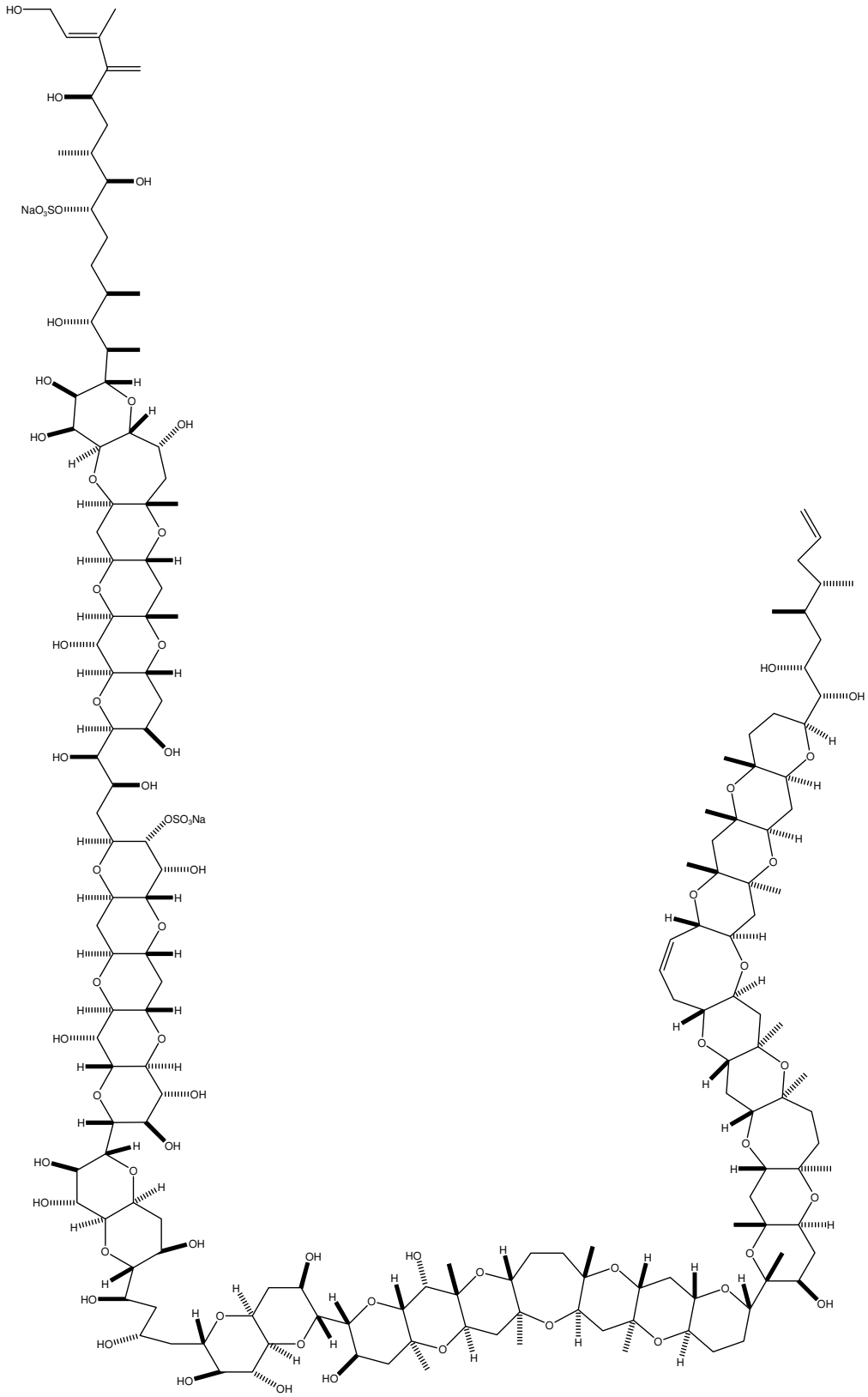


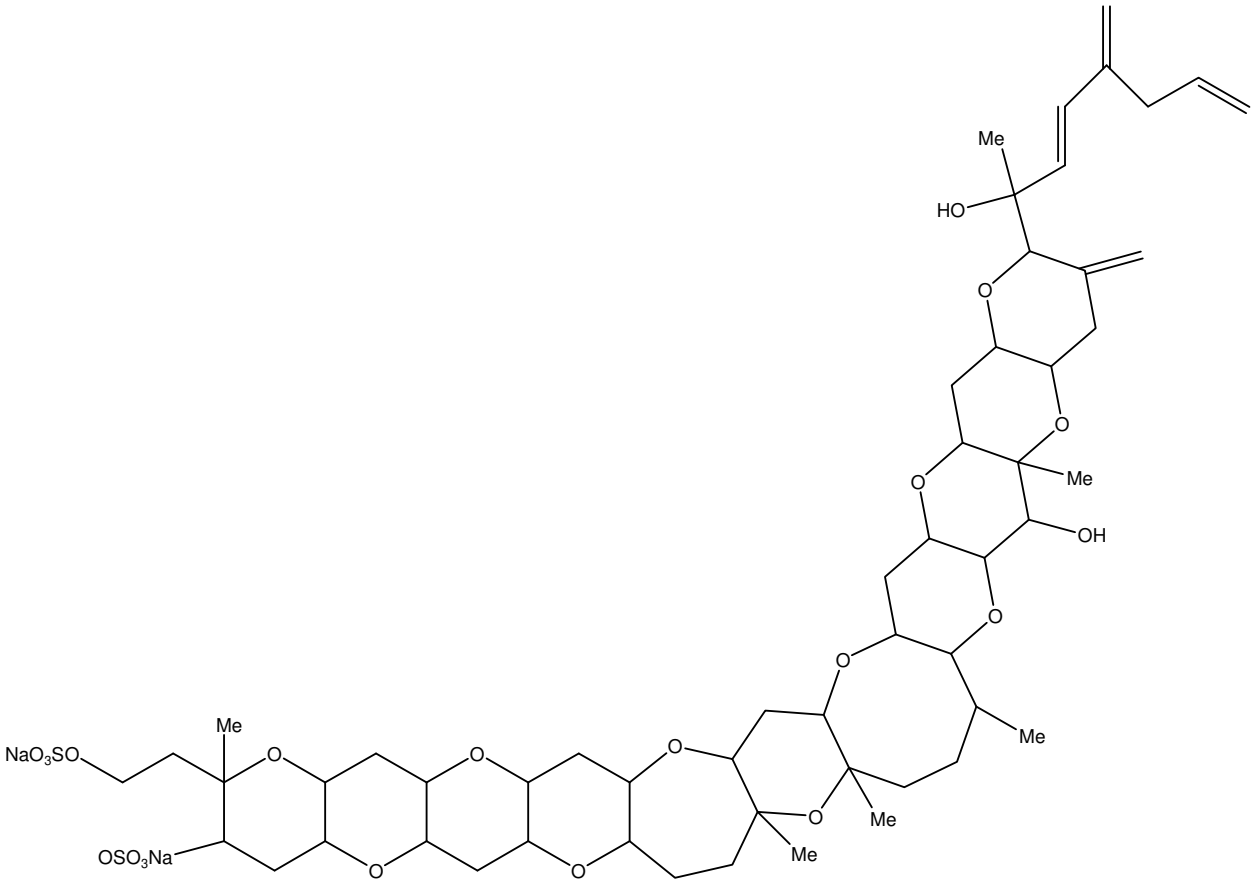
Brevetoxin B **1**



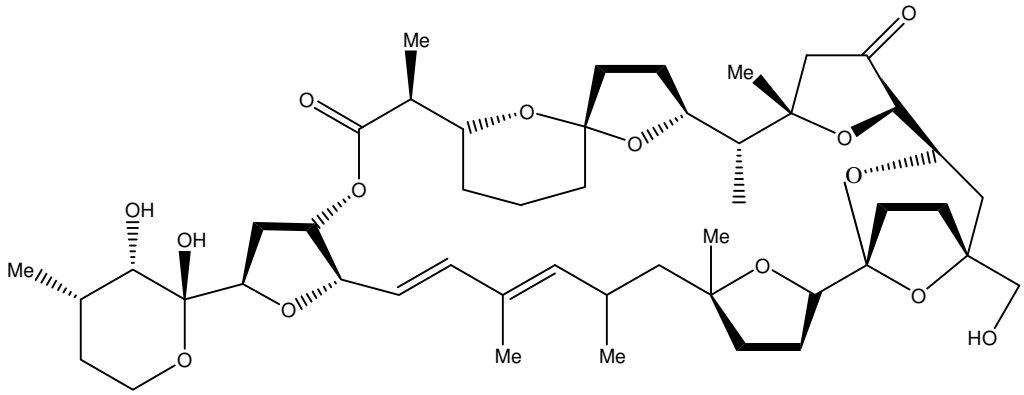
Ciguatoxin **2**

Maitotoxin **3**





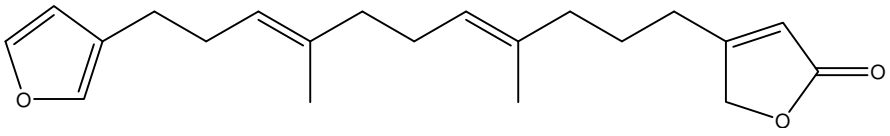
Yessotoxin **4**



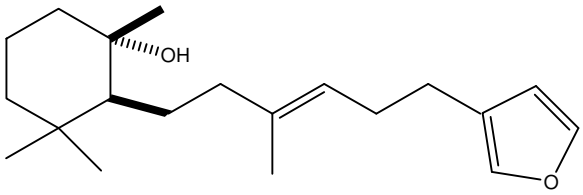
Pectenotoxin-1 **5**

Marine Chemical Ecology

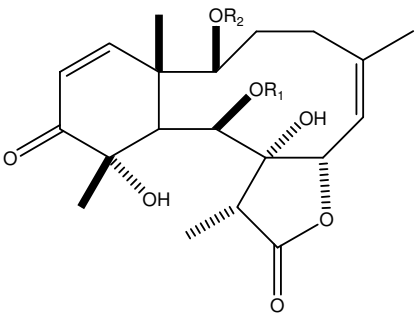
Chemical production in sessile marine organisms helps them to deter predators and also to prevent settling by fouling organisms. A good number of antifoulants has been discovered by natural product chemists as a result of this chemical defense mechanism. Examples include furospongolide **6** [17] and ambiol A **7** [18] from sponges and renillafoulins (**8-10**) from octocoral [19]. Compounds such as stypoldione **11** and latrunculin A **12** from *Stypopodium zonale*[20] and *Latrunculia magnifica* [21] respectively were discovered based on their ichthyoxicity but were later shown to be cytotoxic [22].



Furospongolide **6**



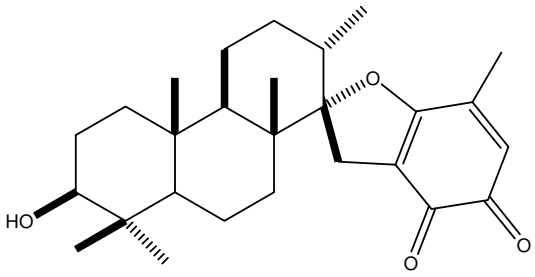
Ambiol A **7**



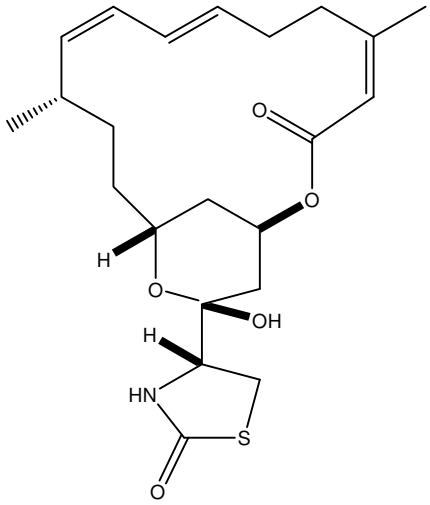
Renillafoulin A **8** R1= R2 = Ac

Renillafoulin B **9** R1= Ac, R2 = C2H5CO-

Renillafoulin C **10** R1= Ac, R2 = n-C3H7CO-



Stypoldione **11**

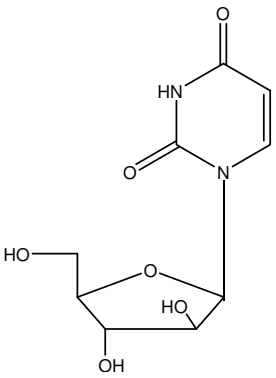


latrunculin A **12**

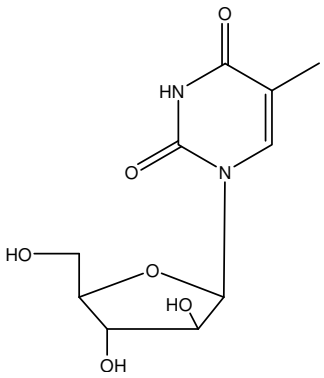
Marine Biomedicinals

This area is concerned primarily with the discovery of bioactive compounds as pharmaceutical agents. Several compounds with antioxidant, antimalaria, antiviral, antibacterial, anti-inflammatory, and anticancer activities have been discovered. The majority of these compounds currently in clinical or preclinical screening are anticancer agents [1].

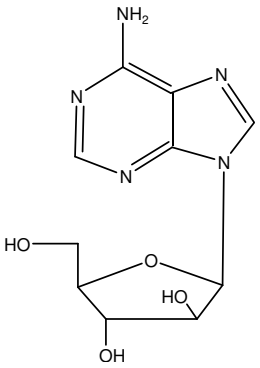
The discovery of the nucleosides spongouridine **13** and spongothymidine **14** from the sponge *Cryptotethya crypta* [23] served as the lead structures for the synthesis of ara-A **15** an antiviral agent [24], and ara-C **16** an anticancer agent for the treatment of leukemia [25].



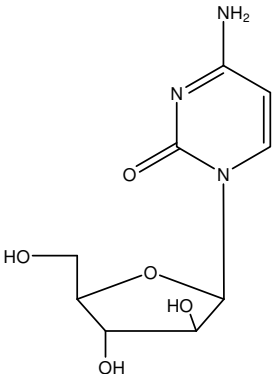
Spongouridine **13**



Spongothymidine **14**

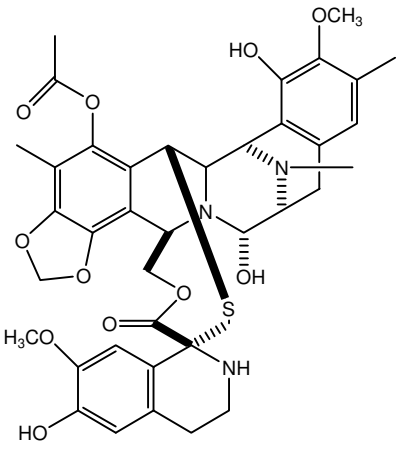


Ara-A **15**

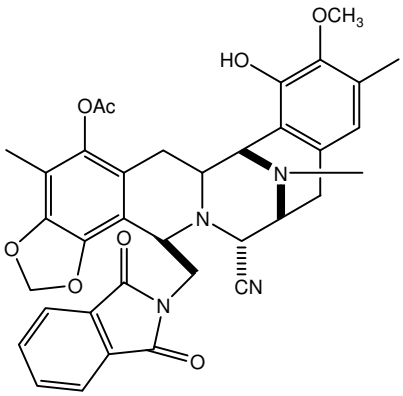


Ara-C **16**

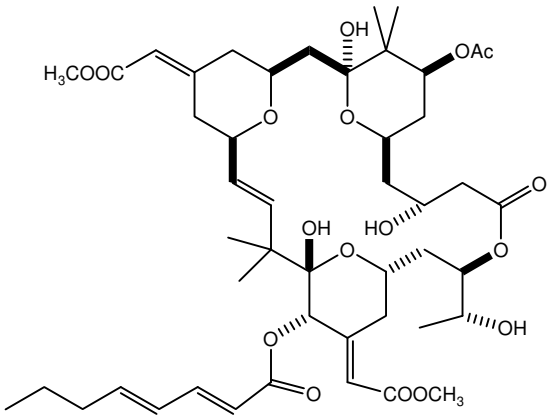
The most advance anticancer drug currently under clinical investigation is the marine alkaloid ecteinascidin 743 **17** isolated from the Caribbean tunicate *Ecteinnascidia turbinate* with its synthetic analogue phthalascidin **18** for the treatment of various types of cancers [6, 26]. Bryostatin 1 **19** isolated from the bryozoan Bugula nertitina [27] is another promising anticancer drug under clinical investigation for the treatment of solid tumors, leukemias, lymphomas, and melanoma [28].



Ecteinascidin 743 **17**



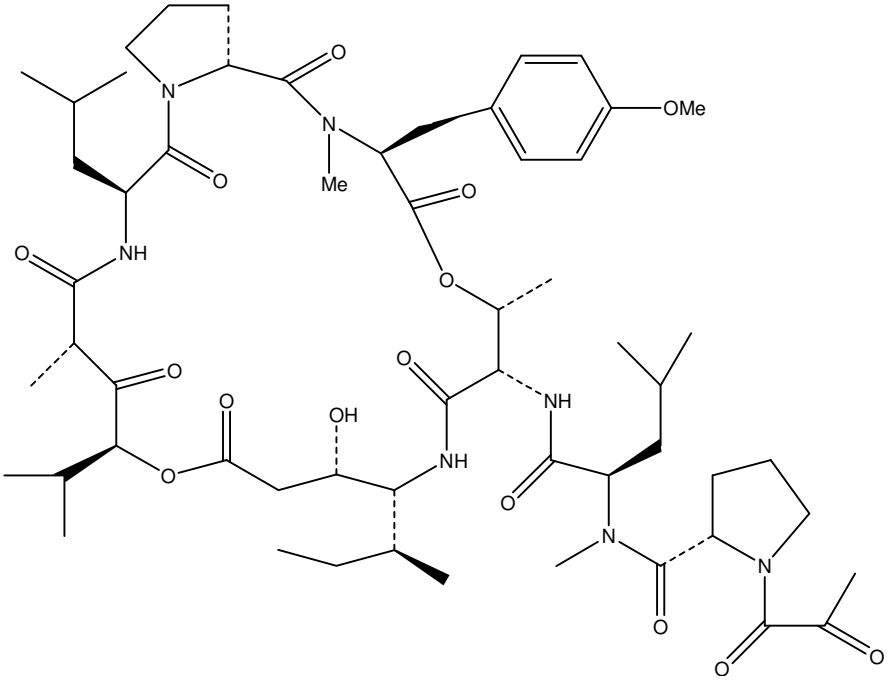
Phthalascidin **18**



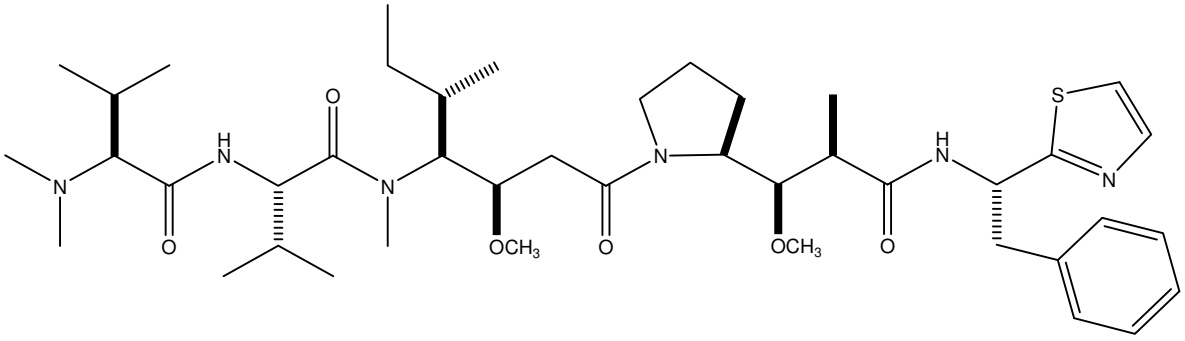
Bryostatin 1 **19**

Other potential anticancer agents currently under clinical investigation include dehydrodidemnin B **20** isolated from the tunicate, *Aplidium albicans* [28], dolastatin 10 **21** from the seahare, *Dolabella auricularia* [29], isohomohalichondrin B **22** from the sponge *Axinella sp* [30], eleutherobin **23** form the soft coral, *Eleutherobia albifora* [31], curacin A **24** from the

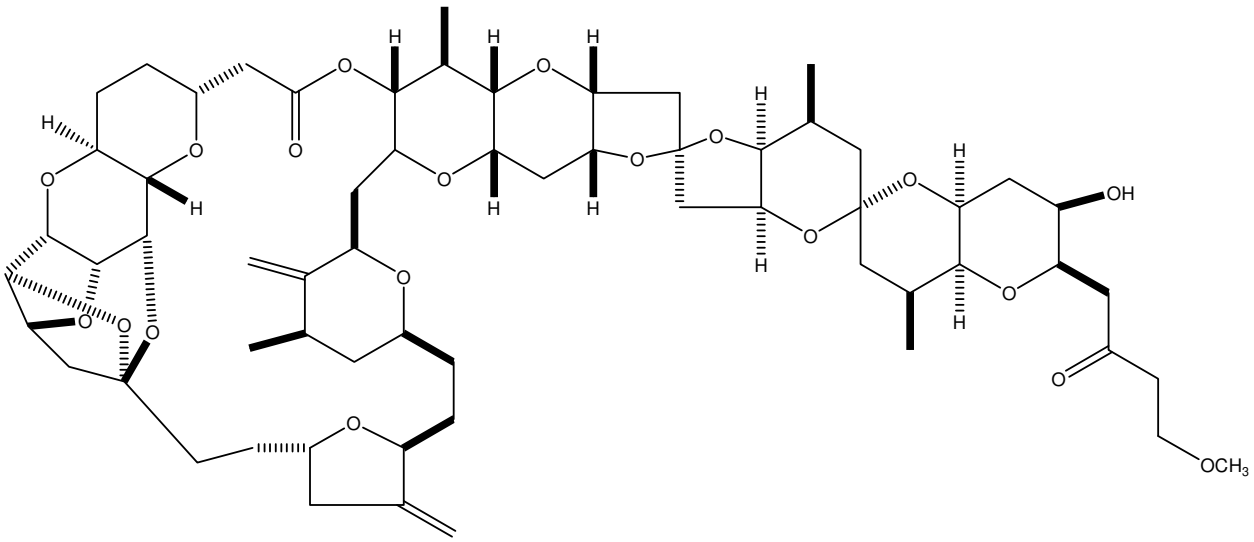
curacao cynobacteria, *Lyngbya majuscule* [32], and kahalalide F **25** from the Hawaiian mollusk, *Elysia* rufescens [33].



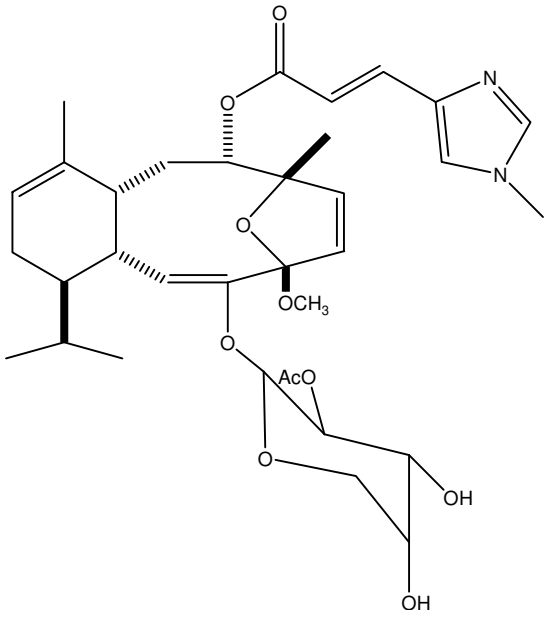
Dehydrodidemnin B **20**



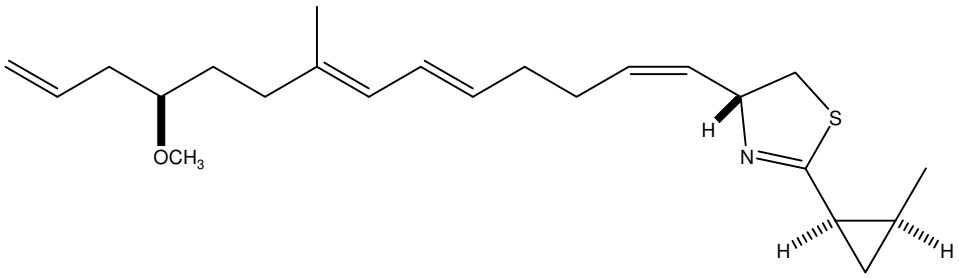
Dolastatin 10 **21**



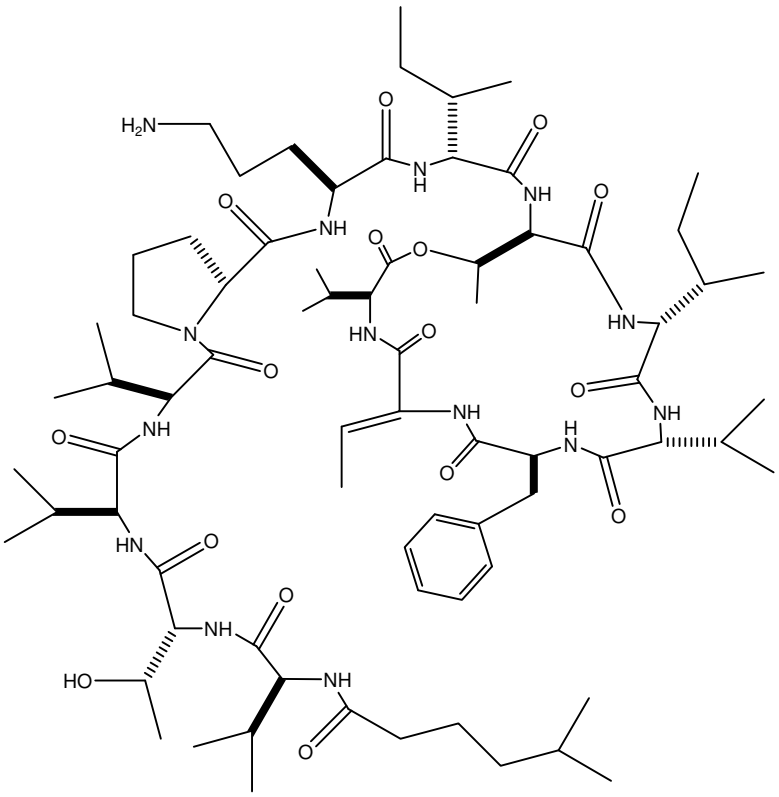
Isohomohalichondrin B **22**



Eleutherobin **23**



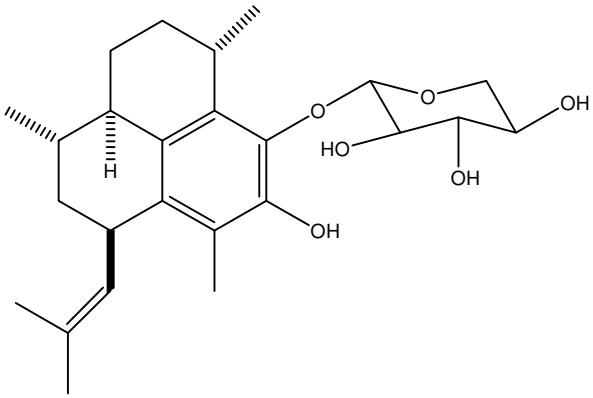
Curacin A **24**



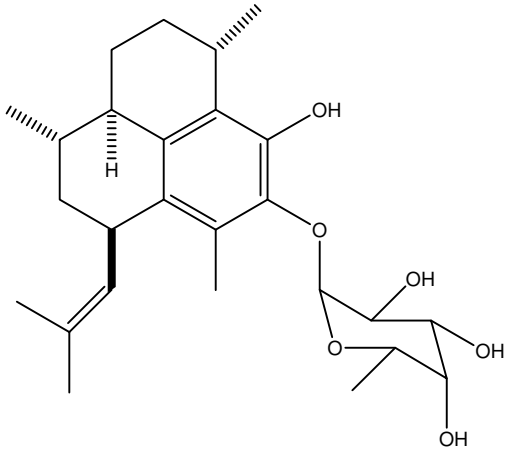
kahalalide F **25**

A good number of anti-inflammatory agents have also been isolated from marine organisms such as pseudopterosins A **26** and E **27** from the Caribbean gorgonian, *Pseudopterogorgia elisabethae* [34, 35], topsentin **28** from the sponge spongosorites ruetzleri

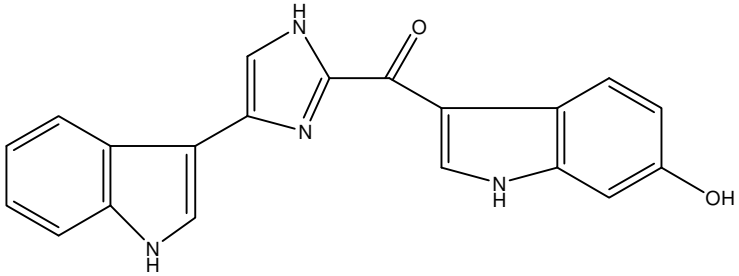
[36], and manoalide **29** from the sponge, luffariella variabilis [37], which has become a standard drug in inflammation research.



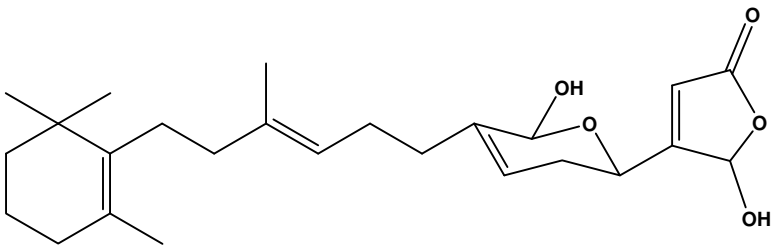
Pseudopterosins A **26**



Pseudopterosins E **27**

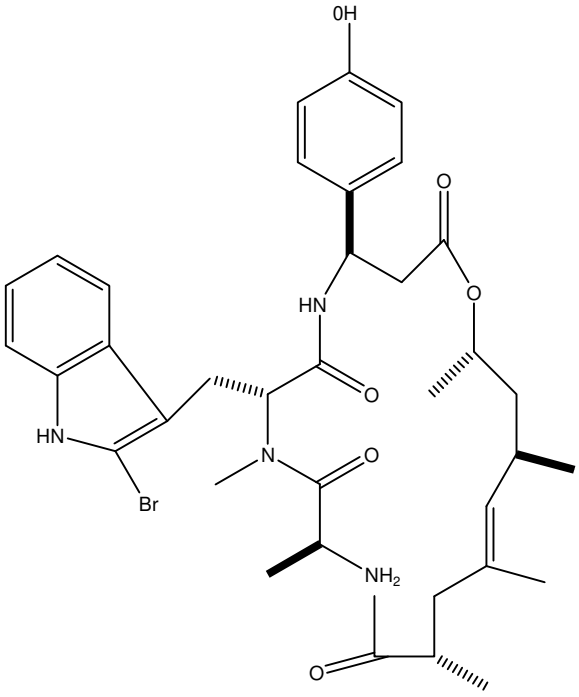


Topsentin **28**

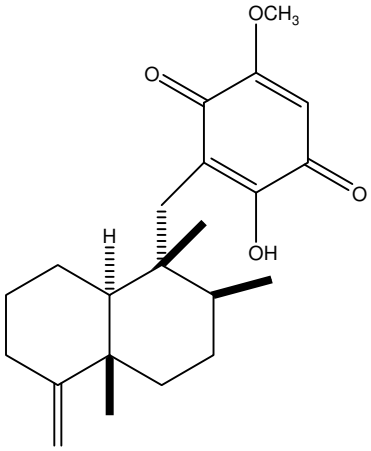


Manoalide **29**

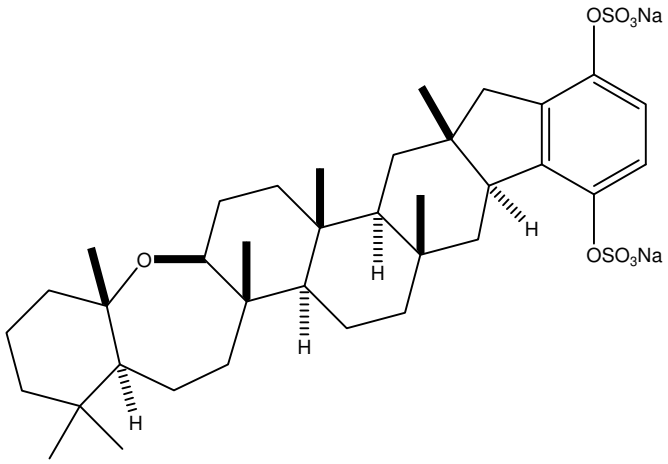
Some compounds are also employed as reagents in cellular biology. Examples include jaspamide **30** [38, 39] that acts on actin, ilimaquinone **31**[40] that causes vesiculation of the golgi apparatus [41], and adociasulfate **32** that is an inhibitor of the intracellular motor protein kinesin [42, 43].



Jaspamide **30**



Ilimaquinone **31**



Adociasulfate **32**

The Role of Secondary Metabolites in Marine Organisms

In general secondary metabolites from any source can be considered as the products of a process of “natural combinatorial chemistry”. This is either because they are the product of genes that have frequently been “shaffled” between taxa or because they are the products of what might be described as “co-metabolism” whereby a molecule is biosynthesized by one organism and then modified by another [44].

The role of secondary metabolites in marine organisms has been a subject of debate for many years. Different views have been generated by two schools of thought on the role of secondary metabolites in producing organisms. The first school of thought postulates that secondary metabolites are waste products that do not play any role in the survival of the organism [45]; to the contrary the second school of thought suggests that secondary metabolites are involve in definite biological activities that enhance the survival of the producing organism [46].

Clearly the precursors for these biosynthetic compounds and the energy involved in these biosynthetic pathways can not be dispensable because these resources can be channeled into the growth and reproduction of the producing organism [47], and as such secondary metabolites must play a role in the survival of the producing organism that is chemical means of defense. Secondary metabolites mediate a wide range of ecological interaction or allelopathic interaction between marine organisms ranging from fouling, competition for space, and recognition of food [48, 49].

Marine Chemicals as Anticancer Drugs

The design and synthesis of chemical drugs used in the treatment of cancer is one of the main objectives of scientists in medicinal chemistry. Cancer continues to be one of the major causes of death worldwide, and it is ranked second to heart diseases in killer diseases in the US, with an estimated 1.3million cases in 2006 and 556,902 deaths for that year. The progress made in reducing the morbidity and mortality of this dreadful disease can only be said to be modest. [50,51,52]. Table 1 shows some of the compounds that have been isolated from marine sources that have shown significant anticancer and antitumor activities.

Table 1: Some Marine Derived Anticancer Compounds [6]

Organism	Group	Metabolite
Ecteinascidia tubinata	Tunicate	ET-743
Aplidium albicans	Tunicate	Aplidine
Elisia rufescens	Mollusc	Kahalilide
Spisula polynyma	Mollusc	ES-285
Micromonospora marina	Actinomycete	Thiocoraline
Bugula neritina	Bryozoan	Bryostatin 1
Portieria hornemannii	Red alga	Halomon
Aplysia kurodai	Sea hare	Aplyronine A
Dolabella auricularia	Sea hare	Dolastatin 10
Crambe crambe	Sponge	Crambescidin-816
Halichondria okadai	Sponge	Halichondrin B
Lissodendoryx spp.	Sponge	Isohomohalichondrin B
Mycale spp.	sponge	Mycapeoxide B
*Tridid*emnum soldium	Tunicate	Didemnin B

The marine pharmacy holds more than 35,000 marine-derived biological samples, with approximately 150 compounds shown to be cytotoxic against tumor cells. Furthermore, approximately 35 compounds have a known mechanism(s) of action for their antitumor effect while 124 marine compounds are yet to be studied for their detailed mechanism of antitumor activity. Out of the 35 antitumor compounds, at least a dozen of them are currently in various phases of human clinical trials for treatment of different cancers [53].

The major demerit associated with pharmacological research involving marine organisms is the extremely scarce availability of biologically active substances for bioassays and therapy and its inherently slow nature. Chemical synthesis offers an alternative to overcome the supply issue as well as accelerating the bioassays and therapeutic processes [54, 55].

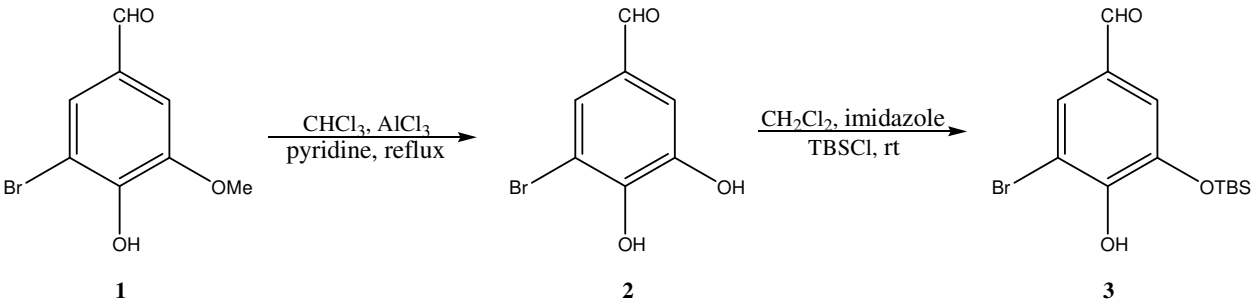
Purpose of this Research

The fundamental goal of this research is to synthesize two marine bioactive compounds; 3-Bromo-4,5-dihydroxybenzaldehyde **2** and 2,3-Dibromo-4,5-dihydroxybenzaldehyde **5** as well as two novel derivatives, 3-Bromo-5-(tert-butyl-dimethyl-silanyloxy)-4-hydroxybenzaldehyde **3** and 1-bromo-2,3-dimethoxy-5-nitrooxymethylbenzene **9**. The biological activity of these compounds on prostate cancer cells will then be examined at the school of medical sciences, ETSU. The commercially available bromovanillin **1** and dibromovanillin **4** will serve as the precursors for all the synthetic compounds.

Compound **2** has been isolated from Polysiphonai urceolata [56] and **5** from Polysiphonia brodiaei [57]. However both **2** and **5** have been isolated from Rhodomela convfervoides [58]. All species belong to the marine red algae family Rhodomelaceae that is known to be rich in bromoine-containing compounds or bromophenols [56].

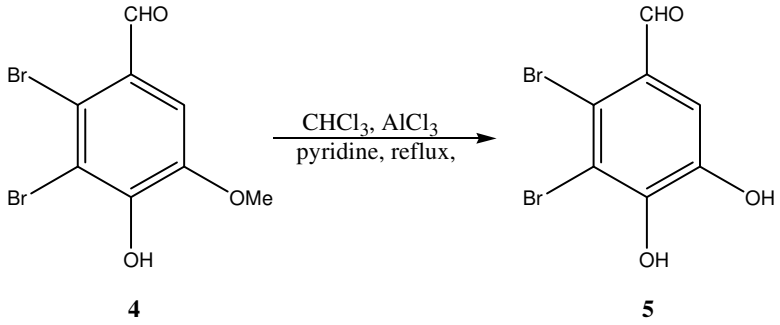
Proposed Synthetic Approach

Synthesis of **2** will be a single step demethylation of bromovanillin **1**, which will then serve as the precursor for the synthesis of our novel derivative **3** by protection of one hydroxyl functional group (Scheme 1)



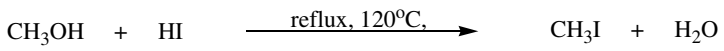
Scheme 1. Proposed Synthetic Pathway for **2** and **3**

Synthesis of compound **5** will follow the same synthetic route as compound **2** that is demethylation of compound **4** (Scheme 2).

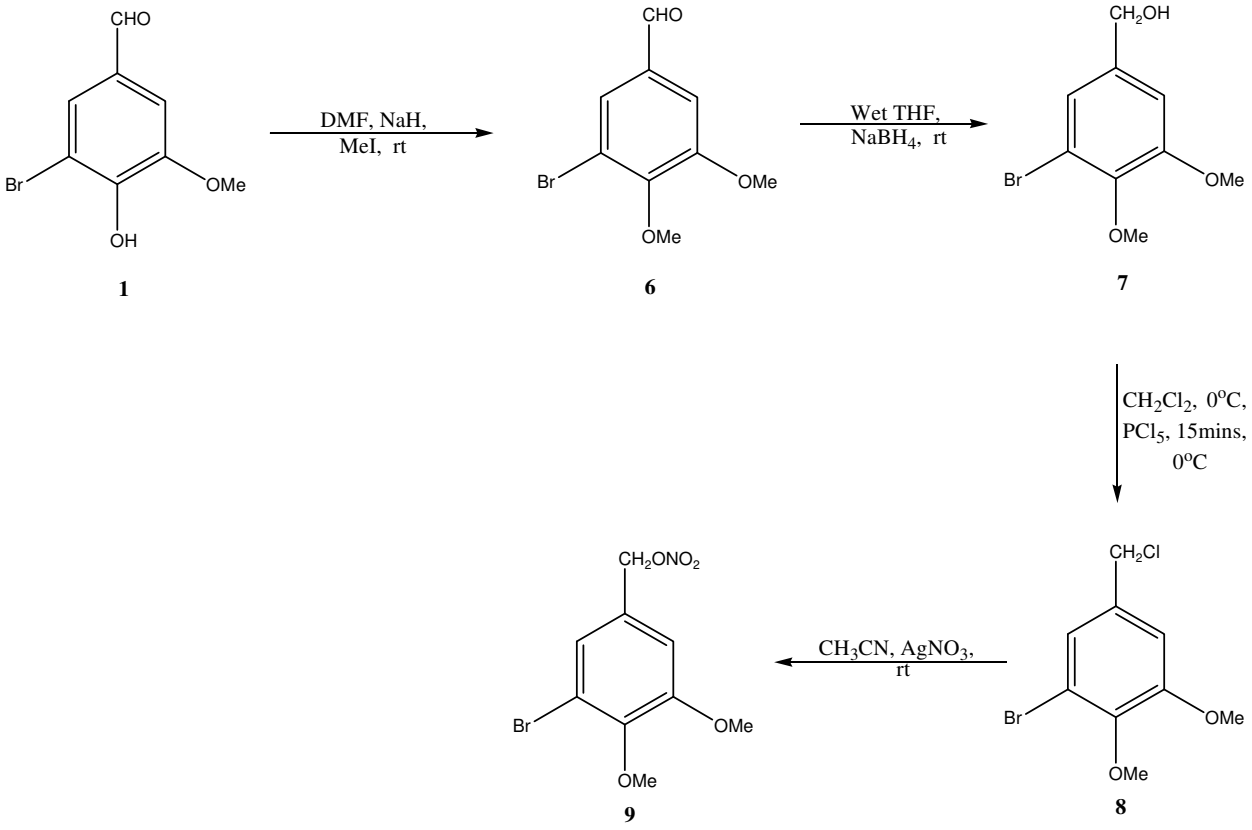


Scheme 2. Proposed Synthetic Pathway of Compound **5**

The synthesis of our second novel derivative begins with the preparation of methyl iodide. This will be achieved by refluxing MeOH in HI (Scheme 3). Next, we will then perform a methylation of **1** to form compound **6** that will then undergo reduction leading to the formation of **7.** Compound **7** will then be chlorinated in an intramolecular S_N2 reaction to obtain compound **8** that will later be nitrated to yield the novel derivative **9** (Scheme 4).



Scheme 3. Preparation of MeI



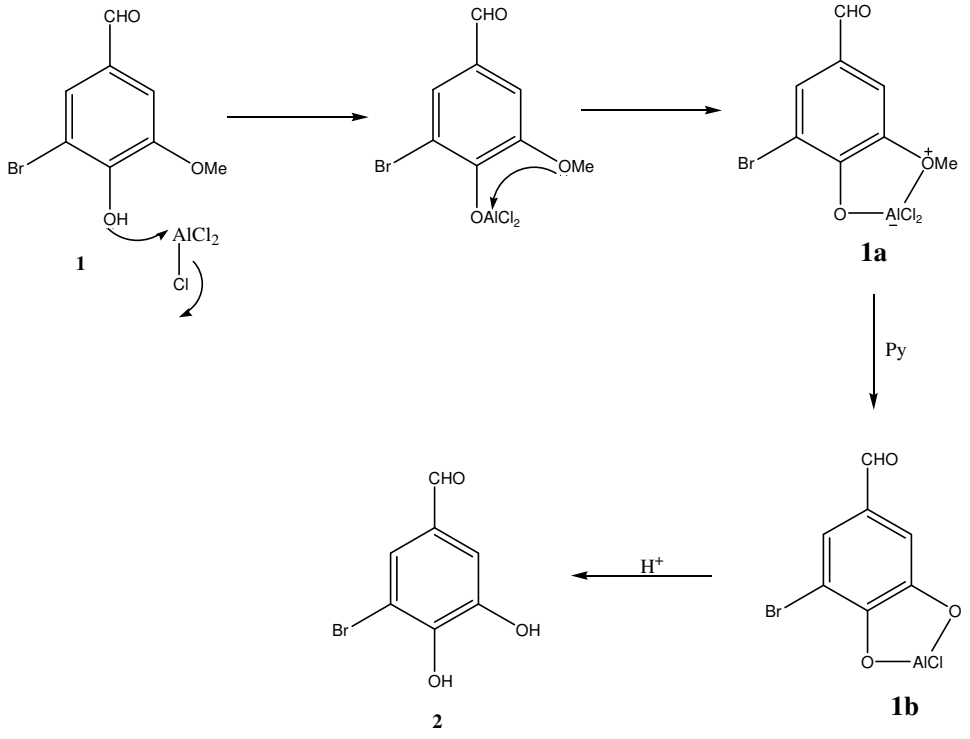
Scheme 4. Proposed Synthetic Pathway of Compound **9**

CHAPTER 2

RESULTS AND DISCUSSION

Synthesis of 3-Bromo-4,5-dihydroxybenzaldehyde **2**

Compound **2** was obtained as a light brown solid after the demethylation of bromo vanillin **1**. The reaction proceeds through a solvated five-member cyclic intermediate **1a** which is attacked by pyridine in nucleophilic displacement reaction resulting in the formation of **1b** that yields compound **2** on hydrolysis (Scheme 5) [59]. A moderate yield of 63% was obtained which is lower than the reported literature yield (83%) [60].



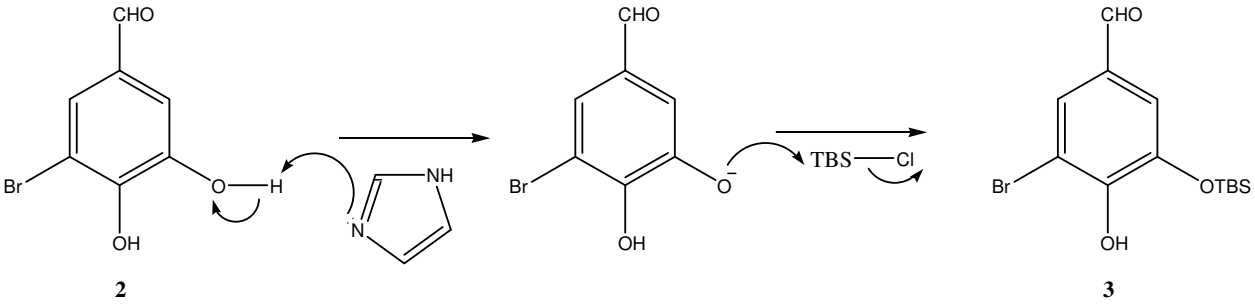
Scheme 5. Mechanism of Demethylation

The biological activity of this compound was carried out on prostate cancer cells using staurosporine as a positive control agent. Compound **2** recorded a 22% inhibition rate and the

positive agent staurosporine recorded a 23% inhibition rate. These results suggest that compound **2** can be effectively compared to the positive control agent staurosporine in terms of efficacy although there is 1% difference between the inhibition rates (Appendix 23)

Synthesis of 3-Bromo-5-(*tert*-butyl-dimethyl-silanyloxy)-4-hydroxybenzaldehyde **3**

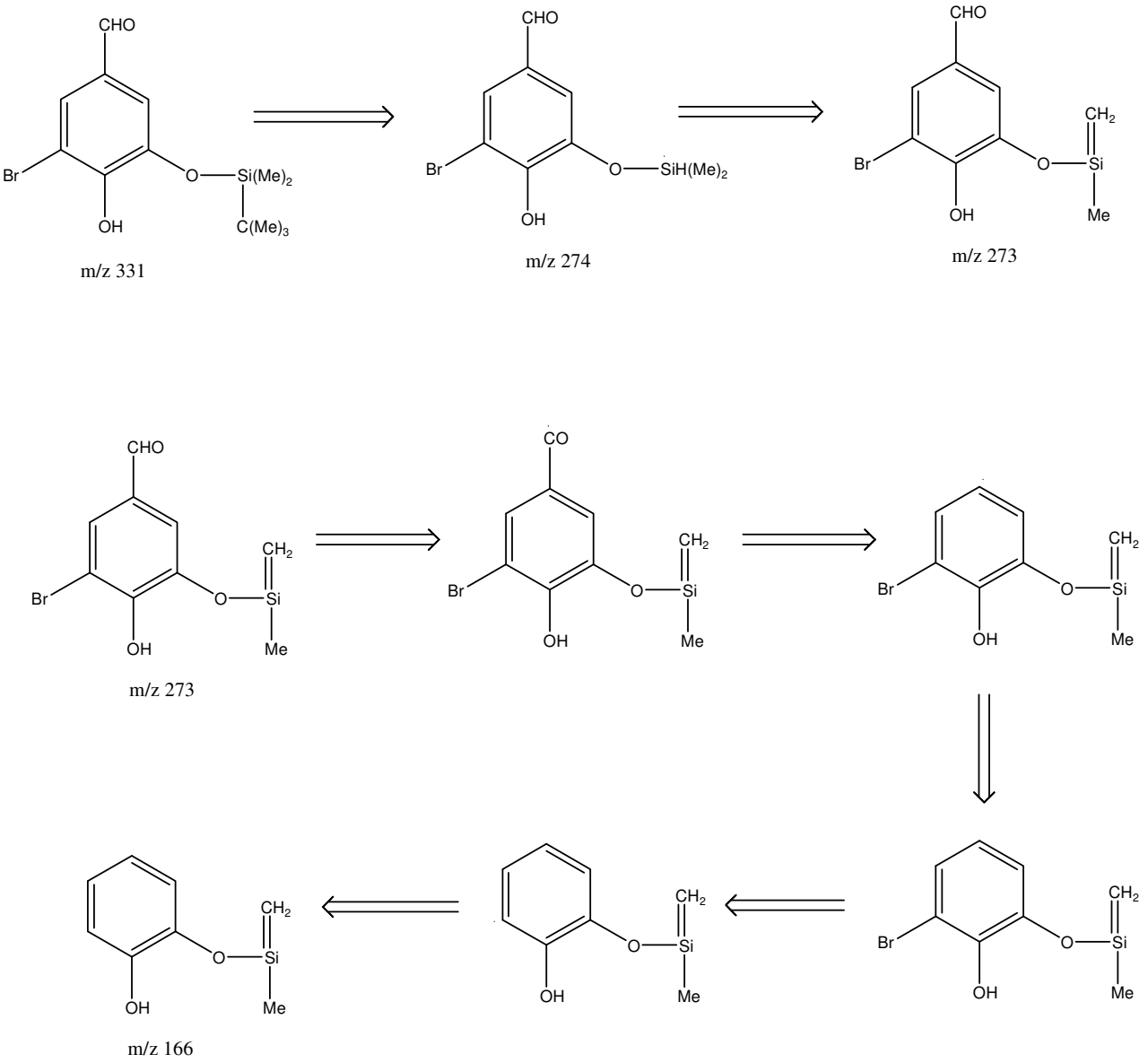
This synthesis was achieved by the modification of a reported literature [61]. Protection of the hydroxy functional group in **2** with a TBS group via an S_N2 reaction mechanism (Scheme 6) afforded compound **3** in 70% yield. This reaction is expected to occur on the hydroxyl group at the meta position due to the bulky nature of bromine (steric effects). It must however be noted that the double protected compound was also isolated in very low yield (15%). Compound **3** was characterized by ^{1}H and ^{13}C NMR, IR, and melting point. (See experimental procedures for data and appendix for spectra.) GCMS analysis of compound **3** exhibited two major peaks at m/z 273 and 166. The proposed fragmentation pattern is shown in Scheme 7.



Scheme 6. Mechanism of Alcohol Protection

Novel compound **3** had a 16% inhibition rate, a decrease of 6% compared to its natural product compound **2** and 7% less than the positive control agent staurosporine. This implies that compound **3** might have a lower efficacy than both the positive control agent

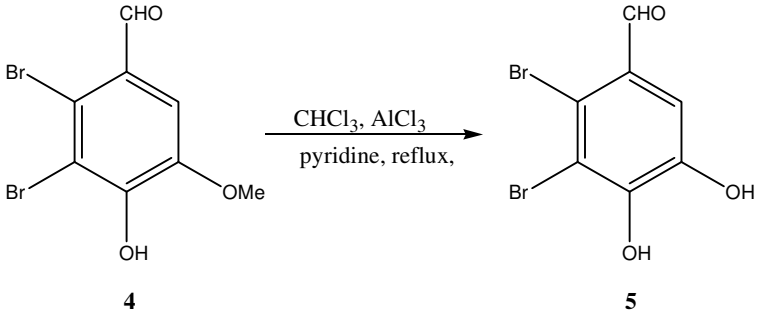
staurosporine and compound **2.** This may be due the substitution of one hydroxy functional group with a TBS group. Introduction of a TBS group on the benzene ring will donate electrons to the ring system and thereby causing a decrease in the reactivity of the carbonyl carbon. The decrease in the inhibition rate of compound **3** can be attributed to the decrease in reactivity of the carbonyl functional group.



Scheme 7. Proposed Fragmentation Pattern of Compound **3**

Synthesis of 2,3-Dibromo-4,5-dihydroxybenzaldehyde **5**

Demethylation of compound **4** afforded compound **5** (Scheme 8) in 63% yield [60]. The product was impure after recrystallization in 50% aqueous EtOH .

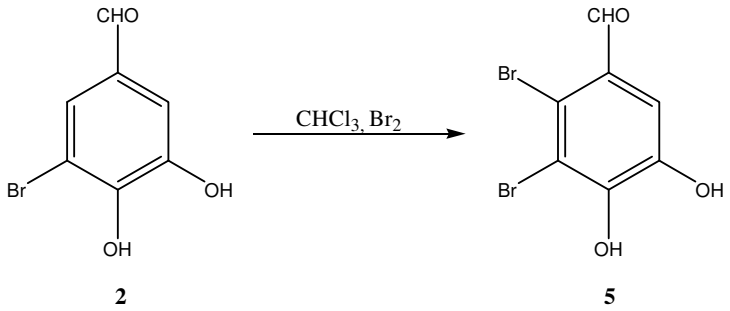


4 **5**

Scheme 8. Synthesis of Compound **5**

This reaction follows the same mechanism as outlined in Scheme 5 [59]. Purification of this compound by column chromatography on silica gel was impossible due to the decomposition of the compound upon contact with silica gel. The recystallization technique was therefore employed to purify the compound. However, purification of this compound was not entirely successful upon using several different solvent combinations. The best solvent combination, 5-10% EtOAc in hexane gave a purity of 84% and a yield of 40%.

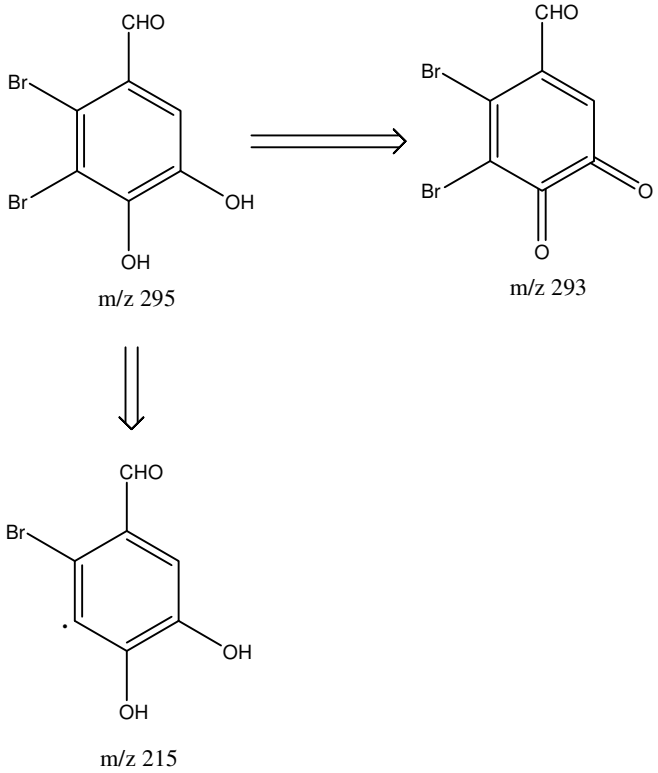
In our attempt to synthesize the pure compound, the starting material was exhausted and an alternate route was designed for the synthesis of compound **5** (Scheme 9) using compound **2** [57] as the precursor due to time constraint.



2 **5**

Scheme 9. Alternate Route for the Synthesis of Compound **5**

Although compound **5** was isolated in the pure form, it was found out that the rate of conversion of compound **2** to **5** was very low (31% yield). The low yield can be attributed to formation of side products such as the isomer of compound **5** and quinone. GCMS analysis of compound **5** exhibited 2 major peaks at m/z 295 and 293 with a minor peak at 215 as shown in the fragmentation (Scheme10)

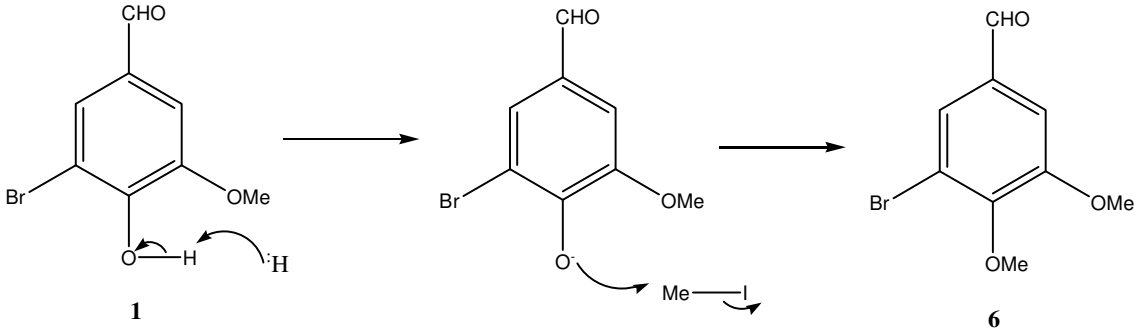


Scheme 10. Fragmentation of Compound **5**

Compound **5** gave the most significant result for biological activity. The observed percentage inhibition rate of prostate cancer cell growth was 35%. That is an increase of 12% more than the positive control agent staurosporine (23%). This can clearly be attributed to the addition of a bromine group to the aromatic ring system. The strong electron withdrawing bromine groups decreases the electron density of the ring system and this may increase the reactivity of the carbonyl carbon.

Synthesis of 3-bromo-4,5-dimethoxybenzaldehyde **6**

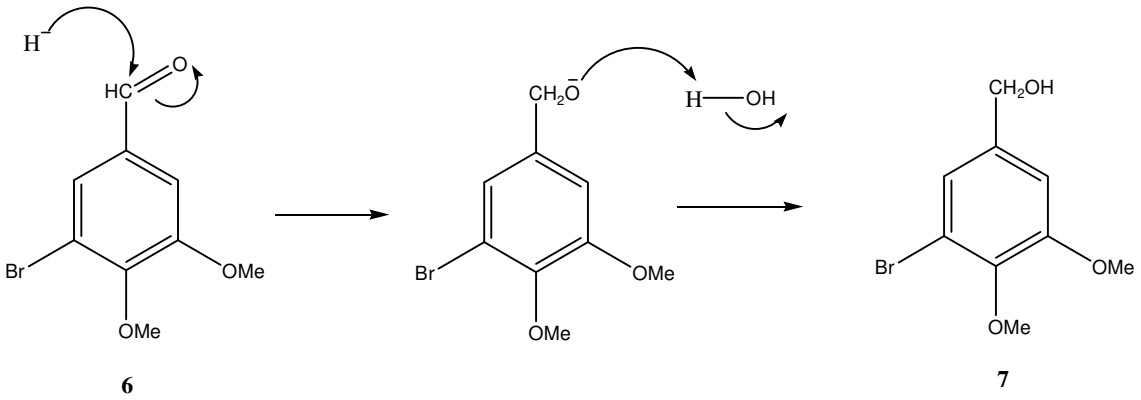
The commercially available bromovanillin **1** served as the precursor for the synthesis of **6** [63]. It was expected that 1eq of NaH should be enough to deprotonate the hydrogen of the alcohol at the para position as shown in the reaction mechanism (Scheme 11). However, upon using 1eq of NaH and 4eq of MeI the yields of the reaction were always less than 45%. We therefore decided to increase the amount of NaH to 2eq which increased the yield to 64%. Further increase in NaH however did not increase the yield of the reaction. The MeI was prepared prior to this reaction and stored at 0^{o}C.



Scheme 11. Mechanism of Methylation

Synthesis of (3-bromo-4,5-dimethoxy-phenyl)-methanol **7**

Compound **7** was synthesized by reduction of **6** with $NaBH_4$ in wet THF using reported literature [63]; however, extension of the reaction time from 5mins to 2 hours and finally to 6 hours increased the yield of the reaction from 46% to 81%. This reaction proceeds by the transfer of a hydride ion from the metal to the carbonyl carbon on the benzene ring. The hydride ion therefore acts as the nucleophile as shown in Scheme 12.



6 **7**

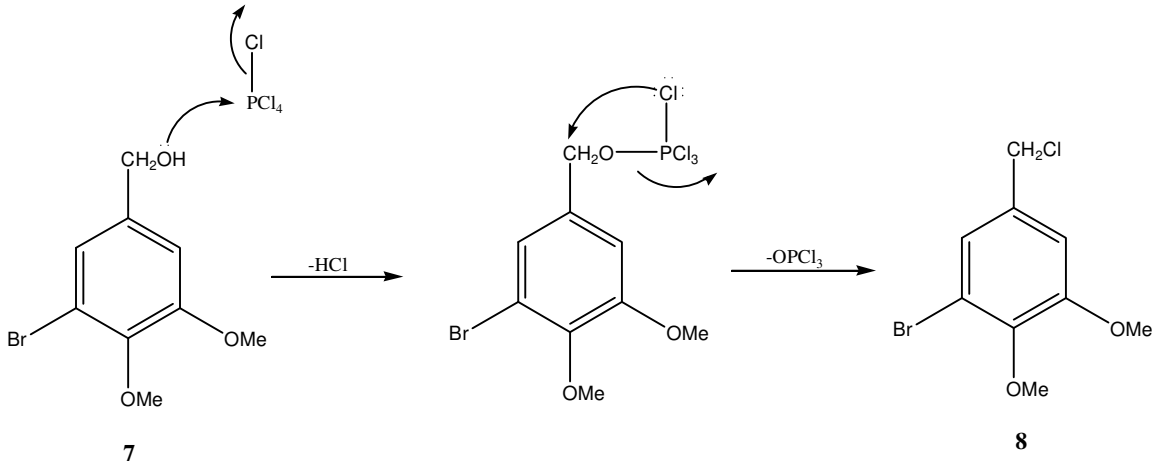
Scheme 12. Mechanism of Carbonyl Reduction

Synthesis of 1-bromo-5-chloromethyl-2,3-dimethoxybenzene **8**

Compound **8** was synthesized by the chlorination of **7** in to yield 81% of a pale yellow liquid [64]. Initially we recorded a 51% yield for this reaction after work-up prior to purification by column chromatography on silica gel. However we recorded a yield of 81% after column purification on silica gel without a work-up.

This reaction proceeds through an S_N2 intramolecular mechanism with a nucleophilic attack of phosphorus by oxygen forming the phosphate ester in the process. An intramolecular

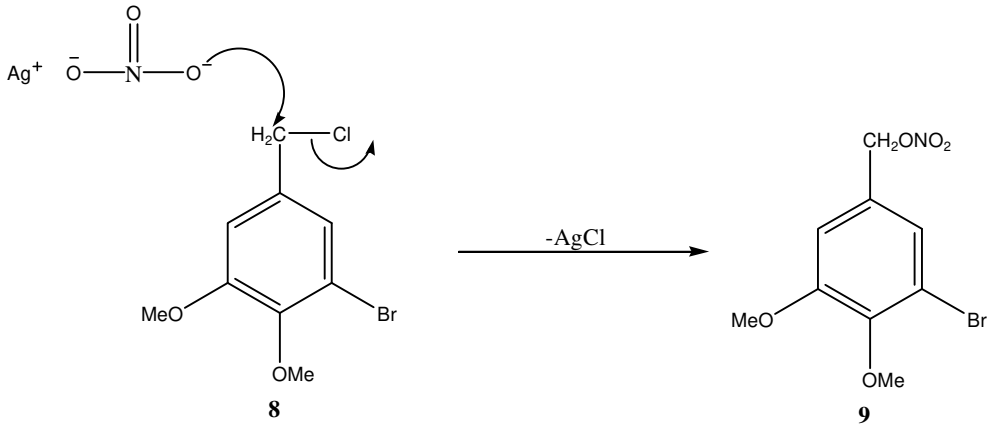
attack of the benzylic carbon by chlorine then liberates the phosphate ester to form compound **8** (Scheme 13).



Scheme 13. Mechanism of Chlorination

Synthesis of 1-bromo-2,3-dimethoxy-5-nitrooxymethylbenzene **9**

Nitration of compound **8** with $AgNO_3$ in CH_3CN for 24hrs afforded compound **9** in 88% [65]. Also, there was no work-up prior to purification by column chromatography on silica gel. The reaction proceeds via an S_N2 mechanism as shown in Scheme 14. This compound was characterized by ^1H and ^{13}C NMR, IR (See experimental procedures for data and Appendix for spectra.) GCMS analysis was not possible due to the decomposition of the compound in the GCMS. The $-ONO_2$ asymmetric stretching vibration was observed at 1625.99 cm^{-1} in the IR analysis which well within the literature value range of 1660-1615 cm^{-1} [66]. Also, this compound is yet to be evaluated for its biological activity.



8 **9**

Scheme 14. Mechanism of Nitration

Inhibition of Cancer Cell Growth

There was a significant reduction in the number of cancer cells after the treatment of the human prostate cell culture with compounds **2**, **3**, and **5** (concentration = 100μM). The reduction in the number of prostate cancer cells by **2**, **3**, and **5** is similar to the positive control agent staurosporin. This indicates the efficacy of the compounds in the inhibition of human prostate cancer cell growth.

Conclusion

The objective of the research has been reached to a large extent with all desired compounds synthesized in high to moderate yields. Compounds **2**, **3**, **5**, and **9** have been synthesized in 64%, 70%, 30%, and 88% yields respectively while the biological activity of **2**, **3**, and **5** were observed at 22%, 16%, and 35% respectively in the inhibition of prostate cancer cell growth (concentration = 100μM). It must be emphasize that the biological activity of compound **9** was not available at the time of writing this report and this was solely due to time constraint.

From the results obtained it can be concluded that compounds **2**, **3**, and **5** are effective in the prevention of prostate cancer cell growth. However, compound **5** is the best among the three compounds.

CHAPTER 3

EXPERIMENTAL

General Methods

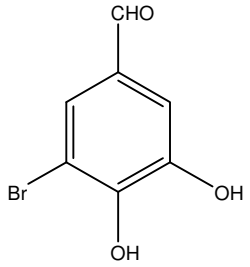
All commercial reagents were used without further purification unless otherwise stated. All proton (^{1}H) and carbon (^{13}C) NMR spectra were recorded on JEOL-NMR Eclipse spectrometer operating at 400MHz and 100MHz for proton and carbon nuclei respectively. Spectra were acquired in $CDCl_3$ unless otherwise stated. Chemical shifts were recorded as delta values in parts per million (ppm) relative to TMS. The multiplicity of signals is reported as follows: s, singlet; d, doublet. Mass spectral analysis was carried out using a Shimadzu GCMS-QP2010 Plus instrument and Infrared spectra were obtained using the Shimadzu IRPrestige-21 FTIR (Fourier Tranformer Infrared Spectrophotometer)

Thin layer chromatography (TLC) was performed with silica gel plate and visualized under a UV fluorescent indicator and column chromatography was carried out on silica gel. All melting points were recorded on Cambridge MEL-TEMP instrument and were not corrected. DMF and THF were distilled over CaH_2 and Na respectively.

Non aqueous extracts were dried with $MgSO_4$ before evaporation in the fume hood and also all organic solvents were evaporated in the fume hood after purification by flash column chromatography unless otherwise stated.

Experimental Procedures

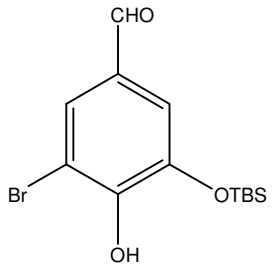
Synthesis of 3-Bromo-4,5-dihydroxybenzaldehyde **2**



2

To a stirred solution of 3-Bromo-4-hydroxy-5-methoxy-benzaldehyde **1** (2.0 g, 8.66 mmol) in chloroform (20mL) cooled in a water bath (5-10 ^{o}C), was added $AlCl_3$ (1.6 g, 12.1 mmol) followed by dropwise addition of pyridine (3.0 mL). The resulting solution was refluxed gently with stirring for 24 hours. The dark solution was then concentrated in vacuo and to the cooled residue was added 3 M HCl till the reaction mixture was acidic. The solid was separated by suction filteration and washed with acid to give after recystallization in aqueous EtOH, 1.21 g (64% yield) of the 3-Bromo-4,5-dihydroxy-benzaldehyde, mp 229-231 ^{o}C, lit. mp 230-232 ^{o}C [60]. ^{1}H NMR (DMSO-D_6 400 MHz, ppm) δ 10.49 (2H, br s, OH); 9.69 (1H, s, CHO); 7.57 (1H, d, *J* = 1.84 MHz, ArH-1); 7.24 (1H, d, *J* = 1.80 MHz, ArH-2). ^{13}C NMR (DMSO-D_6 100 MHz, ppm) δ 191.09, 149.89, 147.07, 129.57, 127.93, 113.29, and 109.98.

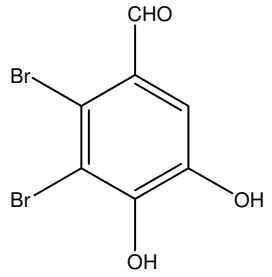
Synthesis of 3-Bromo-5-(tert-butyl-dimethyl-silanyloxy)-4-hydroxybenzaldehyde **3**



3

To a stirred solution of **2** (0.355 g, 1.64 mmol) in CH_2Cl_2 (10 mL) was added imidazole (0.17 g, 2.46 mmol) followed by TBSCl (0.29 g, 1.97 mmol). The resulting solution was stirred for 6 hours at room temperature. Water was then added to terminate the reaction and the mixture was extracted with CH_2Cl_2 (2 x 10 mL) and dried with $MgSO_4$. The solvents were removed by evaporation in the fume hood and purification was done by flash chromatography on a column of silica gel using 3-5% EtOAc in hexane as eluent to afford **3** as a pure white solid (0.38 g, 70%) mp 96-99 ^{o}C. IR (KBr) ν_{max} 3181, 2927, 2861, 1680, 1570, 1500, 1439, 1321, 1255, 1229, 1093, 892 and 782 cm^{-1}. ^{1}H NMR ($CDCl_3$ 400 MHz, ppm) δ 9.74 (1H, s, CHO); δ 7.64 (1H, d, *J* = 1.80 MHz, ArH-2); δ 7.28 (1H, d, *J* = 1.48 MHz, ArH-6); δ 6.32 (1H, s, OH); δ 1.02 (9H, s, C-$(Me)_3$); δ 0.31 (6H, s, Si-$(Me)_2$). ^{13}C NMR ($CDCl_3$ 100 MHz, ppm) δ 189.61, 150.79, 143.77, 130.04, 129.82, 116.03, 108.81, 25.71, 18.31 and -4.28. GCMS (*m/z*) 274, 273, 166,

Synthesis of 2,3-Dibromo-4,5-dihydroxybenzaldehyde **5** - route 1



5

To a stirred solution of **4** (0.5 g, 1.6 mmol) in chloroform (10mL) cooled in a water bath (5-10 ^{o}C), was added $AlCl_3$ (0.32 g, 2.24 mmol) followed by dropwise addition of pyridine 4.5 eq (0.58 mL). The resulting solution was refluxed gently with stirring for 24 hours. The dark solution was then concentrated in vacuo and to the cooled residue was added 3 M HCl till the reaction mixture was acidic. The solid was separated by suction filteration and washed with acid to give after recystallization in aqueous EtOH, 0.31 g (63% yield) of the 2,3-Dibromo-4,5-dihydroxy-benzaldehyde **5**, mp 196-200 ^{o}C, lit. mp 204-206 ^{o}C [57]. ^{1}H NMR (DMSO-D_6 400 MHz, ppm) δ 10.02 (1H, s, CHO); 7.31 (1H, s, ArH). ^{13}C NMR (DMSO-D6 100 MHz, ppm) δ 191.54, 151.43, 145.88, 126.93, 120.69, 114.46 and 114.21. GCMS (*m/z*) 295, 293, 215.

Synthesis of 2,3-Dibromo-4,5-dihydroxybenzaldehyde **5** - route 2

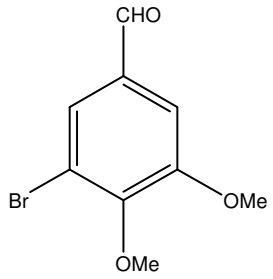
Bromine (0.5 mL, 10.5 mmol) was added dropwise to a stirring and gently refluxing solution of **2** (0.93 g, 4.28 mmol) in chloroform (10 mL). The mixture was stirred under gently reflux for 24 hours after which 10 mL of chloroform was added. The solid that was formed was collected and recystallize in EtOAc to afford 0.38 g of compound **5** (31% yield). mp 199-203 ^{o}C, lit. mp 204-206 ^{o}C [57]. MS (m/z) 295, 293, 265, 215, 187, 159, 131 and 107. ^{1}H NMR (DMSO-D_6

400 MHz, ppm) δ 10.11 (1H, s, CHO); 7.60 (1H, s, ArH). ^{13}C NMR (DMSO-D6 100 MHz, ppm) δ 189.13, 149.17, 143.77, 126.88, 125.42, 113.86 and 109.13. GCMS (*m/z*) 295, 293, 215.

Preparation of Methyl Iodode

MeOH (5 mL, 125 mmol) was added to HI (28 mL) and refluxed for 2 hours in an oil bath at 120^{o}C. The MeI produced was collected in an ice bath at 0^{o}C and dried over CaH_2 to yield 5.8 mL (75%) of pure MeI; ^{1}H NMR ($CDCl_3$ 400 MHz, ppm) δ 2.18 (3H, s, Me).

Synthesis of 3-bromo-4,5-dimethoxybenzaldehyde **6**

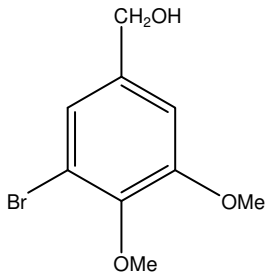


6

To a stirred solution of 3-Bromo-4-hydroxy-5-methoxy-benzaldehyde **1** (1.0 g, 4.33 mmol) in DMF (10 mL) was added NaH (0.21 g, 8.65 mmol) in small portions over 15 minutes. MeI (1.5 mL, 24 mmol) was added after 15 minutes and the resulting solution was stirred for 16 hours at room temperature. The reaction was terminated by the addition of H_2O and the mixture was extracted with hexane (3 x 10mL) and dried with $MgSO_4$. The solvents were removed by evaporation in the fume hood and purification was done by flash chromatography on a column of silica gel using 4% EtOAc in hexane as eluent to afford 0.68 g 0f compound **6** (64%) as a pure white solid, mp 50-53 ^{o}C, lit. mp 51-53 ^{o}C [62]. ^{1}H NMR (DMSO-D_6 400 MHz, ppm) δ 9.88 (1H, s, CHO); 7.78 (1H, d, *J* = 3.32 MHz, ArH-2); 7.54 (1H, d, *J* = 1.84 MHz, ArH-6);

3.88 (3H, s, OMe); 3.84 (3H, s, OMe). ^{13}C NMR ($CDCl_3$ 100 MHz, ppm) δ 189.99, 154.27, 151.88, 133.12, 128.90, 110.12, 60.93 and 56.33.

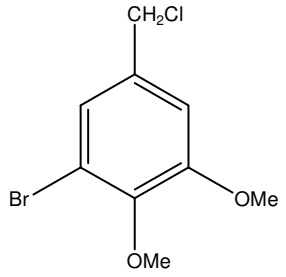
Synthesis of (3-bromo-4,5-dimethoxy-phenyl)-methanol **7**



7

To a stirred solution of **6** (0.52 g, 2.0 mmol) in THF-H_2O (9.7 : 0.3 mL), $NaBH_4$ (0.075 g, 2.0 mmol) was added. The resulting solution was stirred 6 hours at room temperature. Progress of the reaction was monitored by TLC. After completion of the reaction, 10 mL distilled water was added to quench the reaction. The mixture was extracted with EtOAc (3 x 10 mL) and the solvents were removed by evaporation in the fume hood and purification was done by flash chromatography on a column of silica gel using 10-20% EtOAc in hexane as eluent to afford compound **7** as a colorless liquid (0.423 g, 81%). IR (KBr) ν_{max} 3394.72, 2937.59, 1597.06, 1568.16, 1489.05, 1462.04, 1411.89, 1273.02, 1232.51, 1138.0, 1039.63, 999.13, 840.96, 813.96 771.53 665.44 and 445.56 cm^{-1}. ^{1}H NMR ($CDCl_3$ 400 MHz, ppm) δ 7.02 (1H, d, *J* = 1.84 MHz, ArH-1); 6.80 (1H, d, *J* = 1.84 MHz, ArH-6); 4.52 (2H, s, CH_2); 3.80 (3H, s, OMe); 3.77 (3H, s, OMe); 2.69 (1H, s, OH). ^{13}C NMR ($CDCl_3$ 100 MHz, ppm) δ 153.80, 145.69, 138.21, 122.92, 117.60, 110.26, 64.53, 60.68, and 56.12.

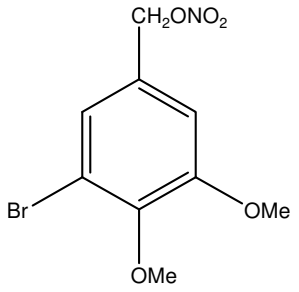
Synthesis of 1-bromo-5-chloromethyl-2,3-dimethoxybenzene **8**



8

To a stirred solution of **7** (0.52 g, 2.1 mmol) in CH_2Cl_2 (5 mL) cooled in a water bath (0-5^oC) was added PCl_5 (0.42 g, 2.0 mmol). The resultant solution was stirred for 15 minutes and the progress of the reaction was monitored by TLC. Flash chromatography on a column of silica gel using 10-20% EtOAc in hexane as eluent was carried out after completion of the reaction to afford compound **8** as a pale yellow liquid (0.456 g, 81%). IR (KBr) ν_{max} 1597.06, 1568.13, 1489.05, 1411.89, 1309.67, 1276.88, 1234.44, 1139.33, 1045.42, 997.2, 815.89, 707.88, and 623.01 cm^{-1} ^1H NMR ($CDCl_3$) δ 7.15 (1H, d, *J* = 1.84 MHz, ArH-2); 6.87 (1H, d, *J* = 0.76 MHz, ArH-6); 4.49 (2H, d, *J* = 0.76 MHz, CH2); 3.86 (6H, d, *J* = 1.74MHz, 2OMe). ^{13}C NMR ($CDCl_3$ 100 MHz, ppm) δ 153.86, 146.64, 134.48, 124.86, 117.66, 112.01, 60.72, 56.22, and 45.55.

Synthesis of 1-bromo-2,3-dimethoxy-5-nitrooxymethylbenzene **9**



9

To a stirred solution of **8** (0.5 g, 1.88 mmol) in CH_3CN (5 mL) was added $AgNO_3$ (0.5 g). The resultant solution was stirred for 24 hours. Flash chromatography on a column of silica gel using 3% EtOAc in hexane as eluent was carried out after completion of the reaction without any work up to afford compound **9** as a pale yellow liquid (0.484 g, 88%). IR (KBr) ν_{max} 1625.99, 1570.06, 1489.05, 1462.04, 1415.75, 1311.59, 1274.95, 1238.30, 1143.79, 1045.42, 997.20, 925.83, 846.75, 819.75, 754.17, 705.95, and 634.58 cm^{-1}. ^1H NMR ($CDCl_3$ 400 MHz, ppm) δ 7.17 (1H, d, *J* = 1.88 MHz, ArH-2); 6.86 (1H, d, *J* = 1.84 MHz, ArH-6); 5.32 (2H, d, *J* = 1.8 MHz, CH_2); 3.86 (6H, d, *J* = 5.48 MHz, 2OMe). ^{13}C NMR ($CDCl_3$ 100 MHz, ppm) δ 154.00, 147.51, 129.26, 125.57, 117.97, 112.29, 73.84, 60.71, and 56.27.

Biological Activity

The prostate cancer cell line (LNCaP) was purchase from the American Type Tissue Collection (ATCC; Manassas, VA). Cells were cultured in RPMI-1640 with phenol red (Invtrogen Corporation, Grand Island, NY) medium containing 10% fetal calf serum (FBS) (Invitrogen Corporation, Grand Island NY) and antibiotics (50 UI penicillin, 50µg/mL streptomycin) (Sigma Chemical Co., St. Louis, MO) at 37^{o}C and 5% CO_2. For the negative control group, only medium (buffer) was added to the cells. For the positive control group, the cells were treated with staurosporine (0.5µM, Sigma Chemical Co., St. Louis, MO). For the NCX 4040 treating group, the cells were treated with NCX 4040 (20µM)

The synthesized marine chemicals and derivatives were tested for their efficacy in inhibition of the growth of the human prostate cancer cell line LNCaP [67-70]. In this assay, the medium (buffer only) was used as a negative control and a known apoptosis inducing drug, staurosporine (0.5µM), was used as a positive control. The inhibiting effect of NCX 4040 on human prostate cancer cell (LNCaP) growth was then determined based on the cell numbers before and after the treatment with the drugs (concentration = 100µM).

REFERENCES

1. Proksch, P.; Edrada, R A; Ebel, R. *Appl. Microbiol. Biotechnol.* **2002**, 59, 125-134.

2. Abad, M.J.; Bermejo, *P. Nat. Prod. Chem.* **2001**, 25, 683-755.

3. Cragg, G.M.; Newman, D.J.; Snader, K.M. *J. Nat. Prod.* **1997**, 60, 52-62.

4. Piggott, A.M.; Karuso, P. *Mar Drugs* **2005**, 3, 36-37.

5. Abreu, P.M.; Branco, P.S. *J.Braz.Chem. Soc.* **2003**, 14, 675-712.

6. Jimeno, J.M. *Anti-Cancer Drugs* **2002**, 13, S15-S16.

7. Diers, J.A.; Ivey, K.D.; El-Alfy, A.; Shaikh, J.; Wang, J.; Kochanowska, A.J.; Stoker, J.F.; Hamann, M.T.; Matsumoto, R.R. *Pharmacology, Biochemistry and Behavior* **2008**, 89, 46-53.

8. Haefner, B. *DDT* **2003**, 8, 536-544.

9. Munro, M.H.G.; Blunt, J.W.; Dumdei, E.J.; Hickford, S.J.H.; Lill, R.E.; Li, S.; Battershill, C.N.; Duckworth, A.R. *Journal of Biotechnology* **1999**, 70 15-16.

10. Blunt, J.W.; Copp, B.R.; Hu, W-P.; Munro, M.H.G.; Northcote, P.T.; Prinsep, M.R. *Nat. Prod. Rep.* **2008**, 25, 35-94.

11. Wright, A. *Methods in Biotechnology* **1998**, 4, 365-408.

12. Lin, Y.-Y.; Risk, M.; Ray, S. M.; Van Engen, D.; Clardy, J.; Golik, J.; James J. C.; Nakanishi, K. *J. Am. Chem. Soc.* **1981**, 103, 6773-6775.

13. Murata, M.; Legrand, A.-M.; lshibashi Y.; Yasumoto, T. *J. Am. Chem. Soc.* **1989**, 111, 8927-8931.

14. Murata, M.; Legrand, A.-M.; lshibashi Y.; Fukui, M.; Yasumoto, T. *J. Am. Chem. Soc.* **1993**, 115, 2060-2062.

15. Nonomura, T.; Sasaki, M.; Matsumori, N.; Miata, M.; Tachibana K.; Yasumoto, T. *Angew. Chem., Int. Ed. Engl.* **1996**, 35, 1675-1678.

16. Bhakuni, D.S.; Rawat, D.S. *Bioactive Marine Natural Products*; Springer New York; **2005**; pp 183-184.

17. Kashman, Y.; Zviely, M. *Experientia* **1980,**36,1279-1279.

18. Walker R P.; Faulkner, D. J. *J. org. chem.* **1981,** 46, 1098-1102.

19. Keifer, P. A; Rinehart, K. L.; Hooper, I. R. *J. org. chem.* **1986,** 51, 4450-4454.

20. Gerwick, W. H.; Fenical, W.; Fritsch N.; Clardy, J. *Tetrahedron Lett.* **1979**, 145-148.

21. Spector, I.; Shochet, N. R.; Kashman Y.; Groweiss, A. *Science,* **1983***,* 219, 493-495.

22. Faulkner, D.J. *Nat. Prod. Rep.* **2000,** 17, 1-6.

23. Bergmann, W.; Feeney, R. J. *J. Org. Chem.* **1951**, 16, 981-987.

24. Lopez, *C.;* Giner-Sorolla, A. *Ann. N.* Y. *Acad. Sci.* **1977**, 284, 351-357.

25. Bodey, G. P.; Freirich, E J.; Monto, R W.; Hewlett, J. S. *Cancer Chemother.* 1969, 53, 59-66.

26. Martinez, E J.; Corey, E J.; Owa, T. *Chern. Biol.* **2001**, 8, 1151-1160.

27. Pettit, G. R; Herald, C. L.; Doubek, D. L.; Herald, D. L.; Arnold, E; Clardy, J. J. *Am. Chem. Soc.* **1982**, 104, 6846-6848.

28. Propper, D. J.; Macaulay, V.; O'Byrne, K. J.; Braybrooke, J. P.; Wilner, S. M.; Ganesan, T. S.; Talbot, D. C.; Harris, A L. *British J. Cane.* **1998**, 78, 1337-1341.

29. Pettit, G. R.; Kamano, Y.; Herald, C. L.; Tuinamn, A. A.; Boettner, F. E; Kizu, H.; Schmidt, J. M.; Baczynskyj, L.; Tomer, K. 8.; Bontems, R. *J. Am. Chem.* Soc. **1987**,109, 6883-6885.

30. Litaudon, M.; Hart, J. B.; Blunt, J. W.; Lake, R J.; Munro, M.H. G. *Tetrahedron Lett.* **1994**, 35, 9435-9438.

31. Lindel, T.; Jensen, P. R; Fenical, W.; Long, B. H.; Casazza, A. M.; Carboni, J.; Fairchild, C. R. *J. Am. chem.* Soc. **1997**, 119, 8744-8745.

32. Gerwick, W. H.; Proteau, P. J.; Nagle, D. G.; Hamel, E; Blokhin, A.; Slate, D. L. *J. Org. Chem.* **1994**, 59, 1243-1245.

33. Hamann, M. T.; Scheuer, P. J. *J. Am. Chem. Soc*. **1993**, 115, 5825-5826.

34. Look, S. A; Fenical, W.; Matsumoto, G. K; Clardy, J. *J. Org. Chem.* **1986***,* 51, 5140-5145.

35. Roussis, V.; Wu, Z, Fenical, W.; Strobel, S. A.; Van Duyne, G. D.; Clardy, J. *J. Org. Chem.* 1990,55,4916-4922.

36. Bartik, K; Braekman, J. C.; Daloze, D.; Stoller, C.; Huysecom, J.; Vandevyver, G.; Ottinger, R. *Can. J. Chem.* **1987**, 65, 2118-2121.

37. De Silva, E D.; Scheuer, P. J. *Tetrahedron Lett.* **1980**, 21, 1611-1614.

38. Zabriskie, T. M.; Klocke, J. A.; Ireland, C. M.; Marcus, A. H.; Molinski, T. F.; Faulkner, D. J.; Xu C.; Clardy, J. *J. Am. Chem. Soc.* **1986***,* 108, 3123-3124.

39. Crews, P.; Manes L. V.; Boehler, M. *Tetrahedron Lett.* **1986**, 27, 2797-2800.

40. Luibrand, R. T.; Erdman, T. R.; Vollmer, J. J.; Scheuer, P. J.; Finer J.; Clardy, J. *Tetrahedron* **1979**, 35, 609-612.

41. Takizawa, P. A.; Yucel, J. K.; Viet, B.; Faulkner, D. J.; Deerinck, T.; Soto, G.; Ellisman M., Malhotra, V. *Cell* **1993**, 73, 1079-1090.

42. Blackburn, C. L.; Hopmann, C.; Sakowicz, R; Berdelis, M. S.; Goldstein, L.S. B.; Faulkner, D. J. *J. Org. Chem.* **1999,** 64, 5565-5570.

43. Sakowicz, R.; Berdelis, M. S.; Ray, K.; Blackburn, C. L.; Hopmann, C.; Faulkner D.J.; Goldstein, L. S. B. *Science* **1998**, 280, 292-295.

44. Newman, D.J.; Cragg, G.M. *Current Drug Targets* **2006**, 7, 279-304.

45. Davies, J. *Mol. Microbiol.* **1990**, 4, 1227-1232.

46. Stone, M. J.; Williams, D. H. *Mol. Microbiol.* **1992,** 6, 29-34.

47. Herms, D. A; Mattson, W. J. *Quart. Rev. Biol.* **1992,** 67, 283-335.

48. Leflaive, J.; Ten-Hage, L. *Freshwater Biology* **2007**, 52, 199-200.

49. Nys, R.D.; Dworjanyn, S.A.; Steinberg, P.D. *Mar. Ecol. Prog. Ser.* **1998**, 162, 79-87.

50. Xie, W.; Ye, Y.; Shen, A.; Zhou, L; Lou, Z.; Wang, X,; Hu, J. *vibrational spectroscopy* **2008**, 47, 119-123.

51. Hail, N. J.; Cortes, M.; Drake, E. N.; Spallholz, J. E. *Free Radical Biology and Medicine* **2008,** 45, 97-110.

52. Am Cancer Society, Cancer Facts and figures **2006**.

53. Arif, J.M.; Al-Hazzani, A.A.; Kunhi, M.; Al-khodairy, F. *J. of Biomed. And Biotech.* **2004,** 2, 93-

98.

54. Mancini, I.; Defant, A.; Guella, G. *Anti Feeding Agent in Medicinal Chemistry* **2007**, 6, 17-48.

55. Faulkner, D.J. *Marine Pharmacology* **2000**, 77, 135-145.

56. Kurata, K.; Amiya, T. *Bull. Chem. Soc. Jpn.* **1980**, 53, 2020-2022.

57. Lundgren, L.; Olsson, K.; Theander, O. A*cta Chemica Scandinavica* **1979**, B 33, 105-108.

58. Xu, N.; Fan, X.; Yan, X.; Tseng, C.K. *J. of Applied Phycology***2004**, 16, 451-456.

59. Lange, R.G. *J. Org. Chem. Soc.* **1962**, 27, 2037-2039.

60. Vallejos, G.; Fierro, A.; Rezende, M.C.; Sepulveda-Boza, S.; Reyes-Parada, M. *Bioorganic and Medicinal Chemistry* **2005**, 13, 4450-4457.

61. Kihara, N.; Motoda, S.; Yokozawa, T.; Takata, T. *Organic Letters* **2005**, 7, S1-S6.

62. Pettit et al, *J. Nat. Prod.* **2005**, 68, 1450-1458.

63. Zeynizadeh, B.; Behyar, T. *Bull. Chem. Soc. Jpn.* **2005**, 78, 307-315.

64. Kantevari, S.; Narasimhaji, V.; Mereyala, H.B. *Tetrahedron* **2005**, 61, 5849-5854.

65. Hulsman et al, *J. Med. Chem.* **2007**, 50, 2424-2431.

66. Socrates, G. *Infrared and Raman Characteristic Group Frequencies Tables and Charts* 3rd Ed; **2004**, 195-196.

67. Kassi, E.; Papoutsi, Z.; Pratsinis, H.; Aligiannis, N.; Manoussakis, M.; Moutsatsou, P. *J. Cancer Res. Clin. Oncol*. **2007**, *133*, 493-500.

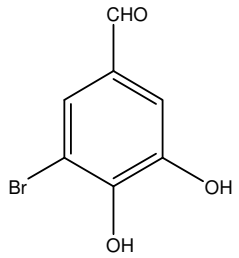
68. Malikova, J.; Zdarilova, A.; Hlobilkova, A.; Ulrichova, J. *Cell Biol.Toxicol.* **2006***, 22*, 439-453.

69. Ahmad, S.; Ahmad, A.; Schneider, K.; White, C*. Int. J. Toxicol.* **2006***, 25,* 17-23.

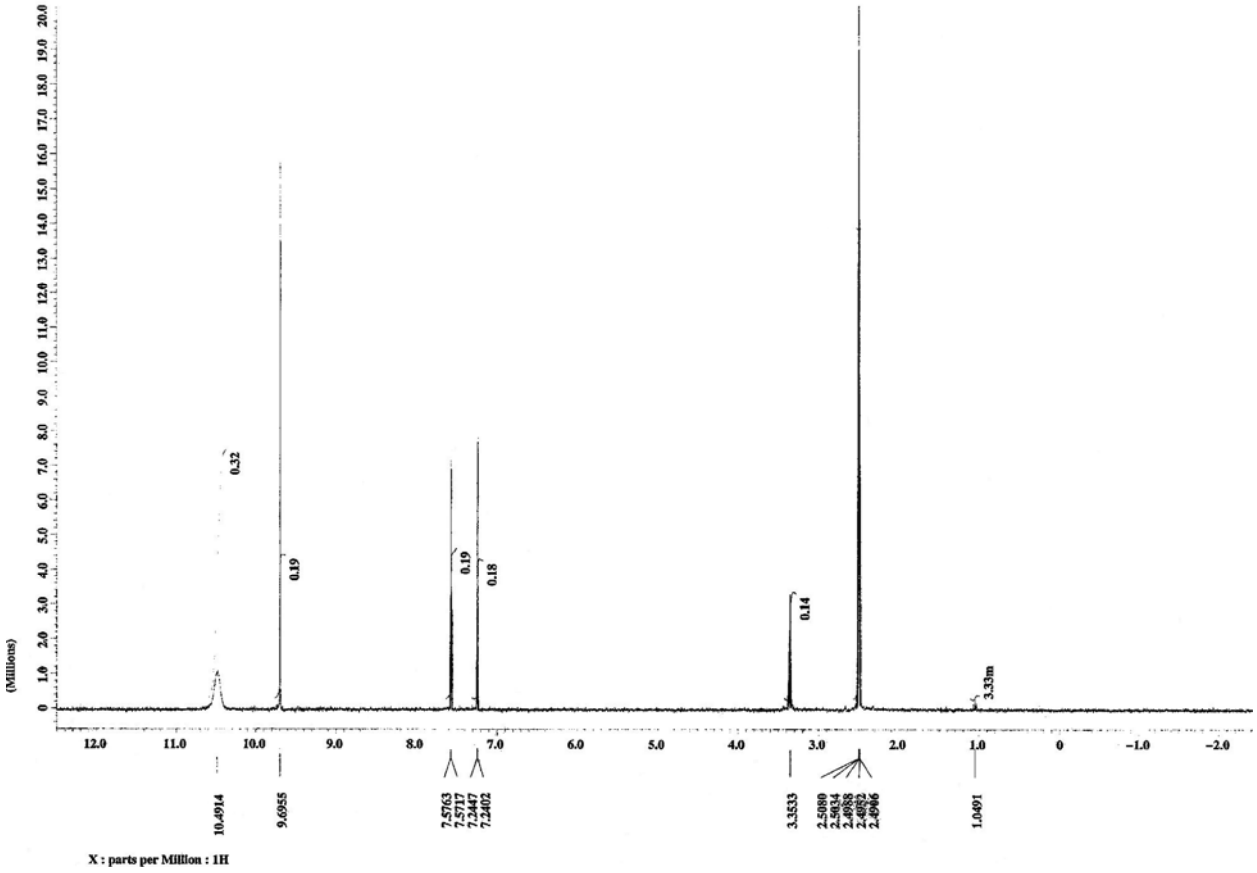
70. Zhang, X.; Ling, M.-T.; Wang, X.; Wong, Y.C. *Int. J. Cancer* **2006***, 118,* 2072-2081**.**

APPENDICES

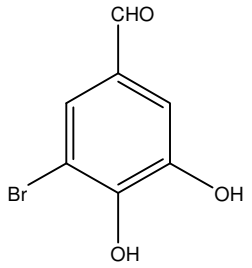
APPENDIX A. ^{1}H NMR Spectrum of Compound **2** in DMSO-d_6



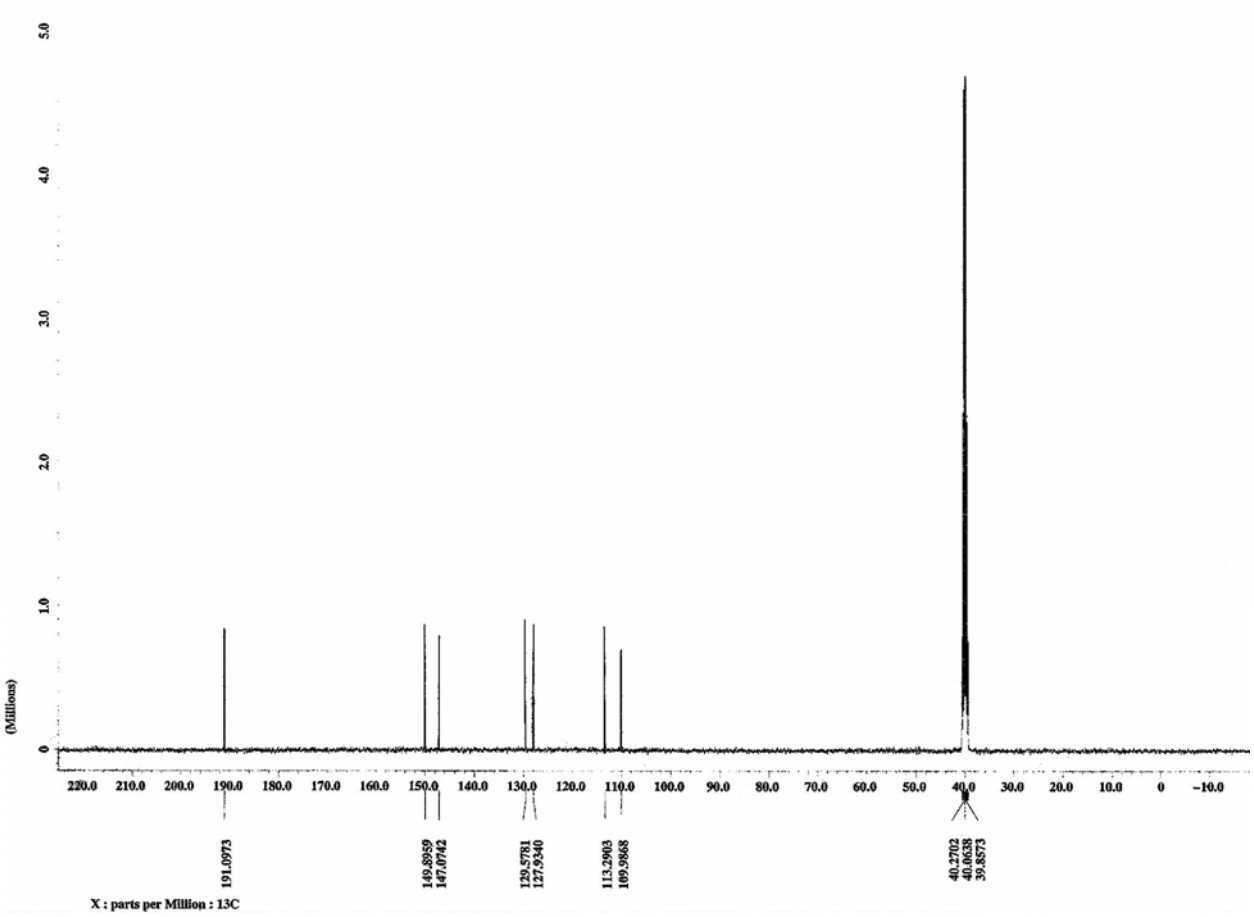
2



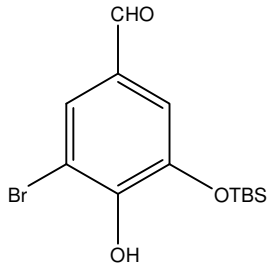
APPENDIX B. ^{13}C NMR Spectrum of Compound **2** in DMSO-d_6



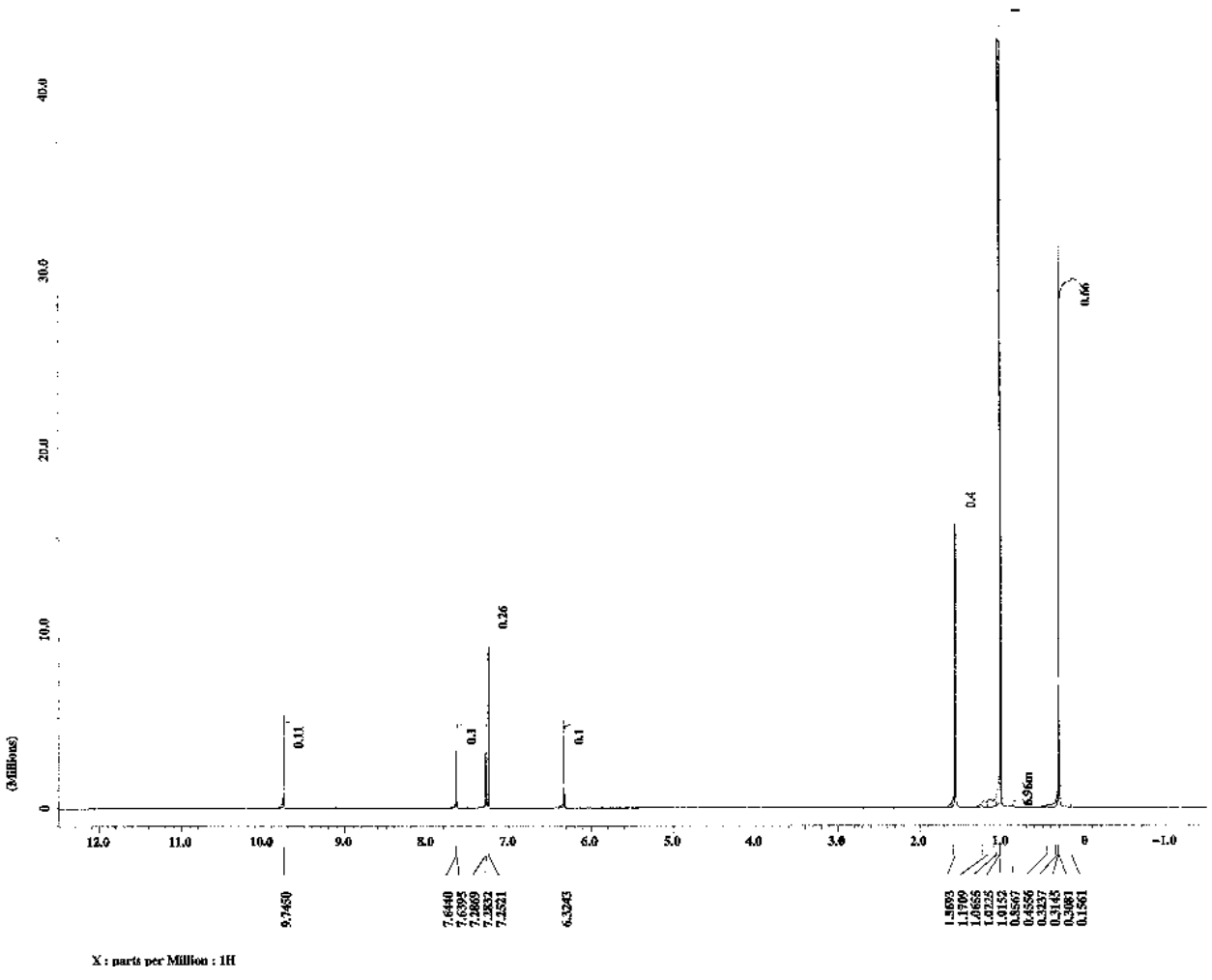
2



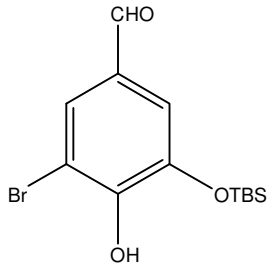
APPENDIX C. ^{1}H NMR Spectrum of Compound **3** in $CDCl_3$



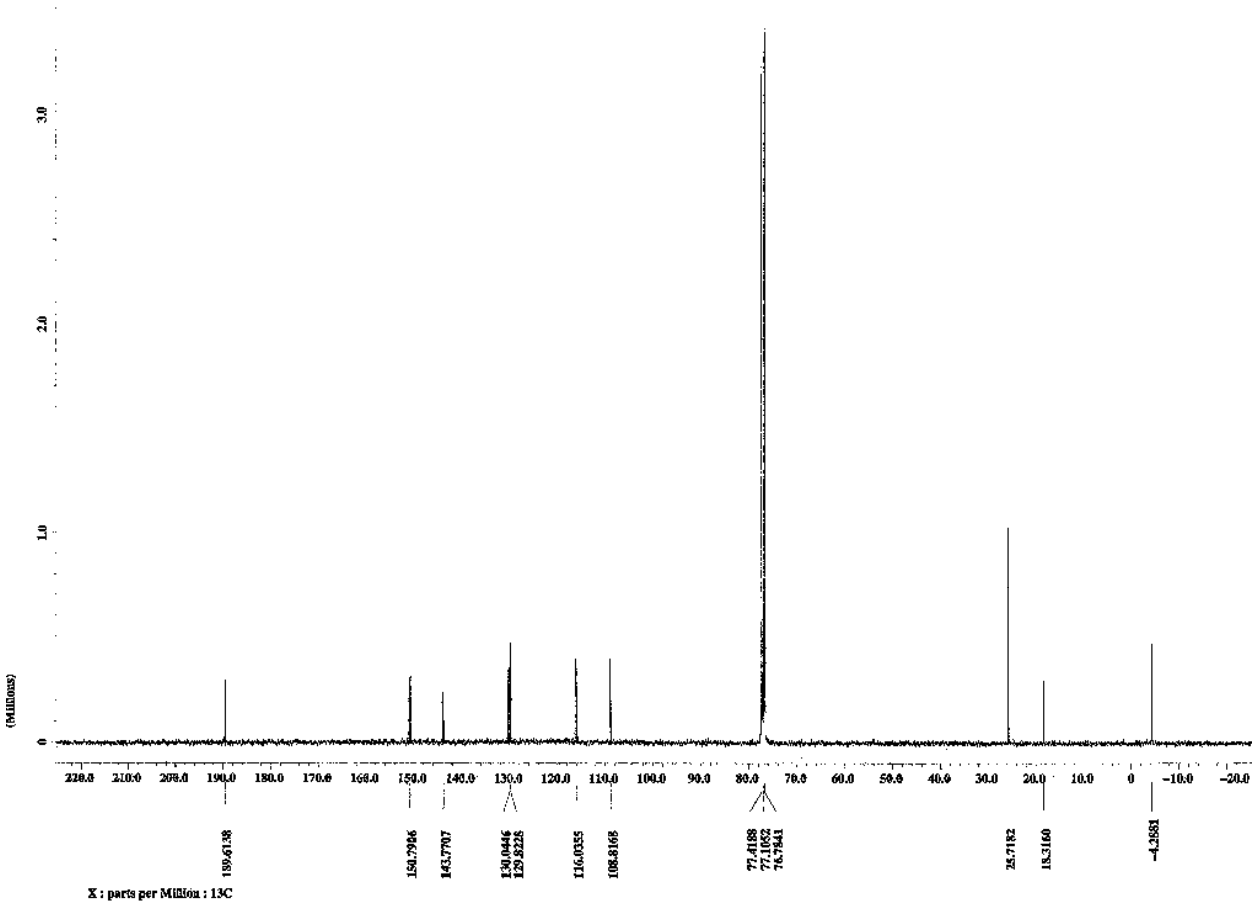
3



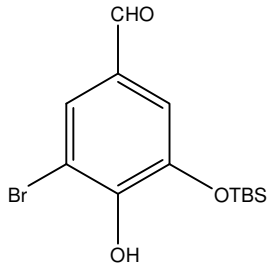
APPENDIX D. ^{13}C NMR Spectrum of Compound **3** in $CDCl_3$



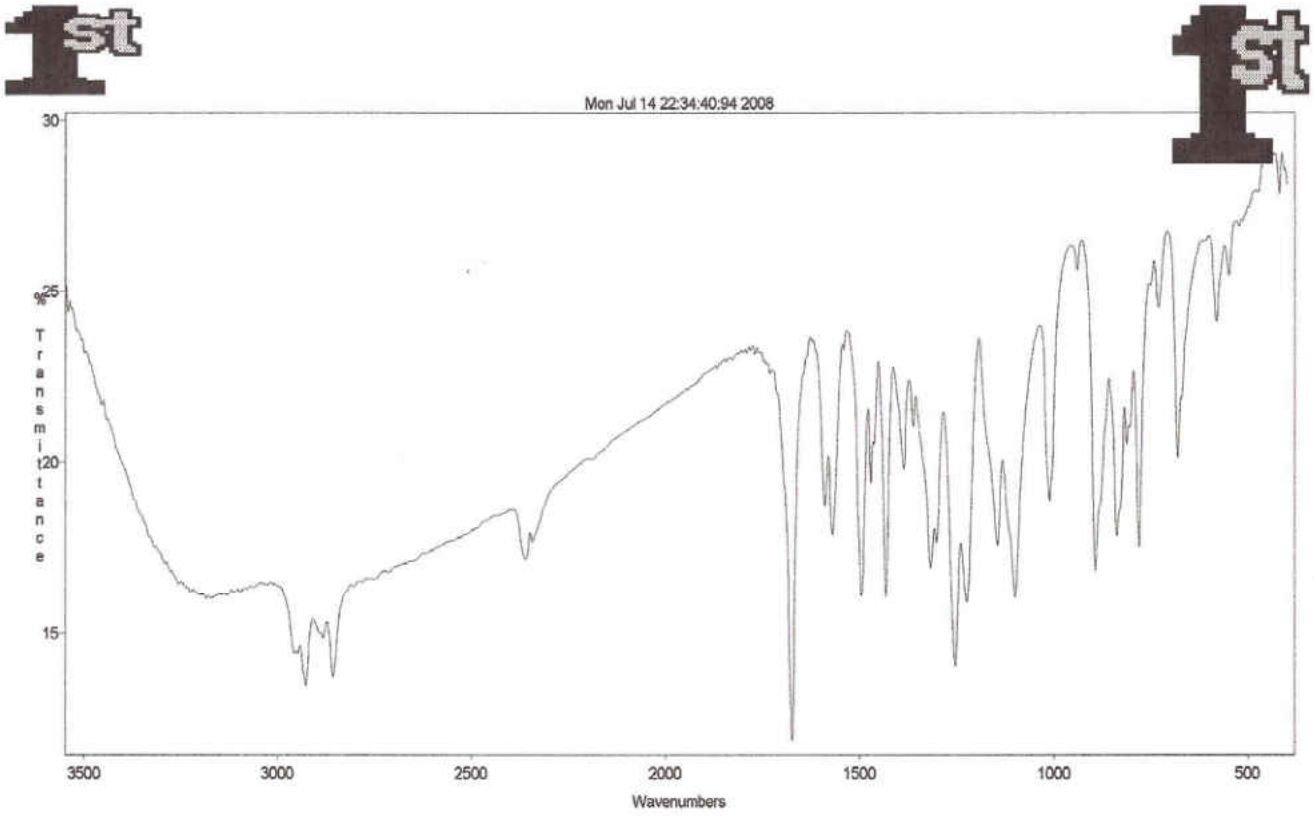
3



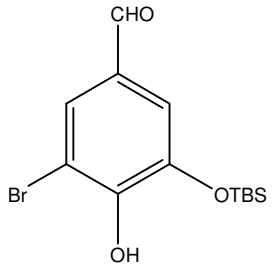
APPENDIX E. IR Spectrum of Compound **3**



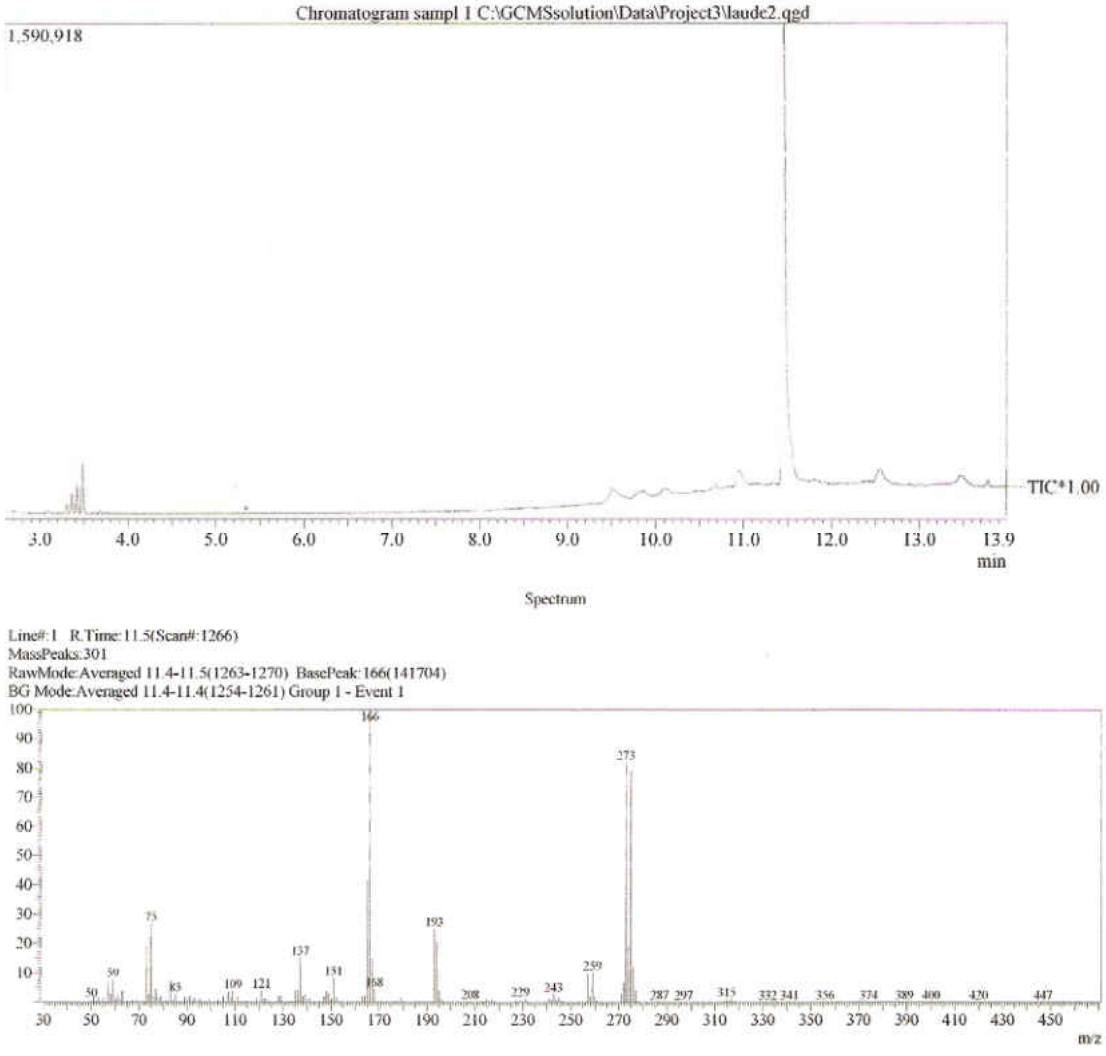
3



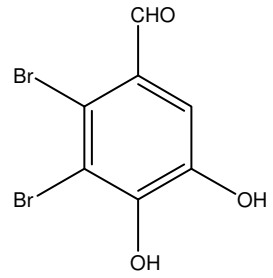
APPENDIX F. GCMS Spectrum of Compound **3** in acetone



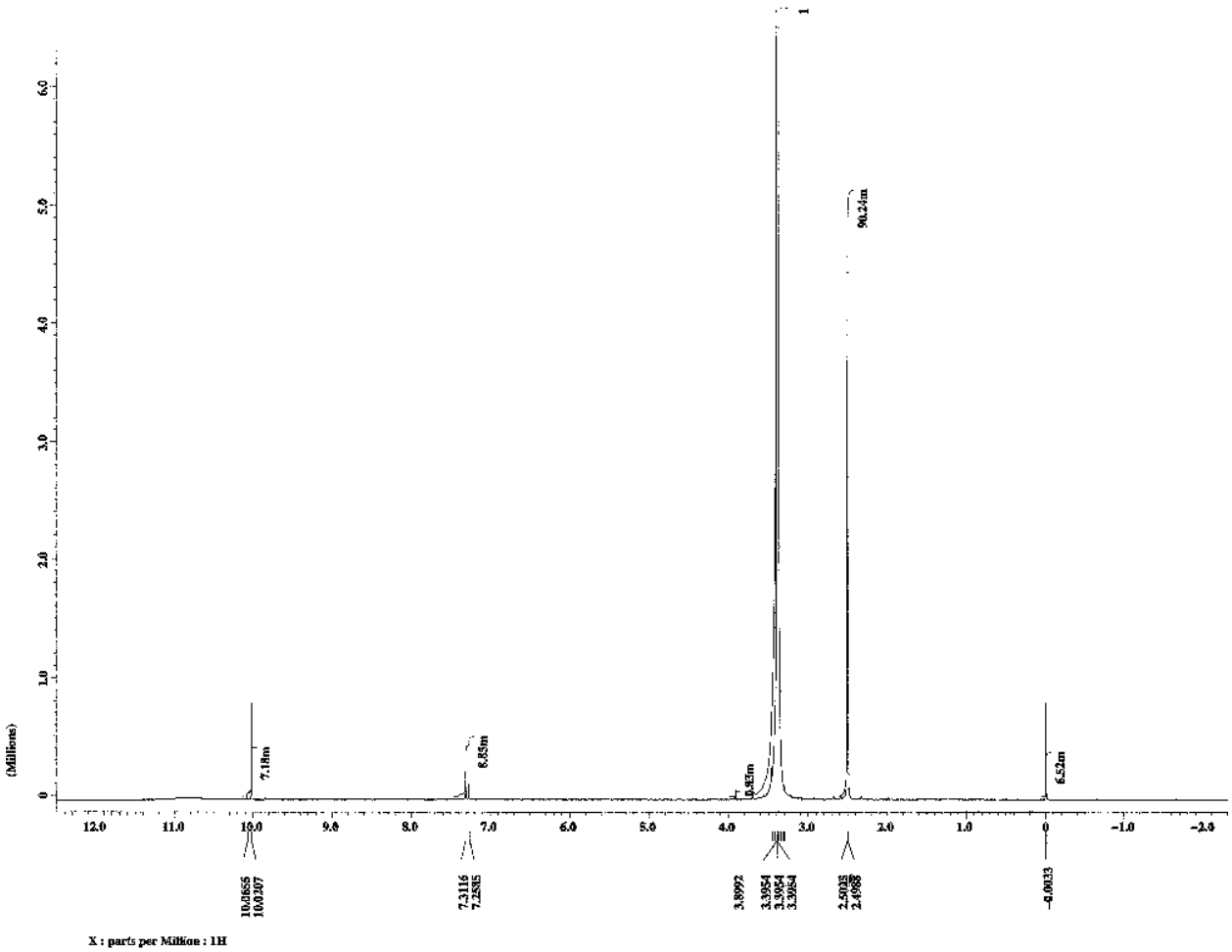
3



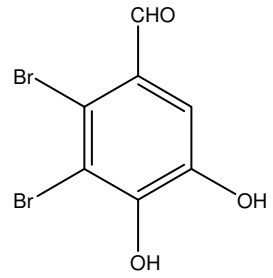
APPENDIX G. ^{1}H NMR Spectrum of Compound **5** in DMSO-d_6 – route 1



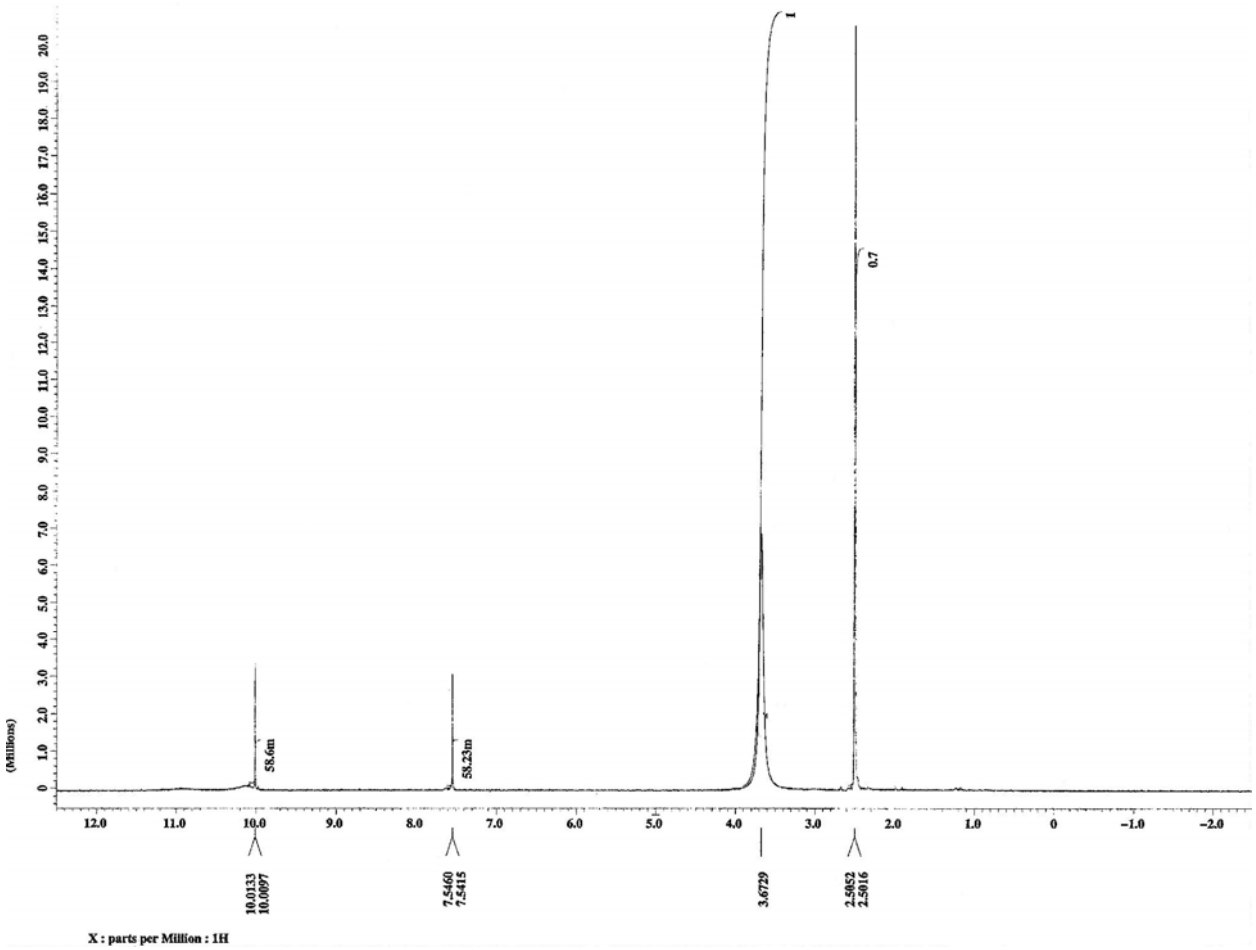
5



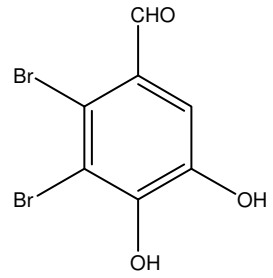
APPENDIX H. ^{1}H NMR Spectrum of Compound **5** in DMSO-d_6 – route 2



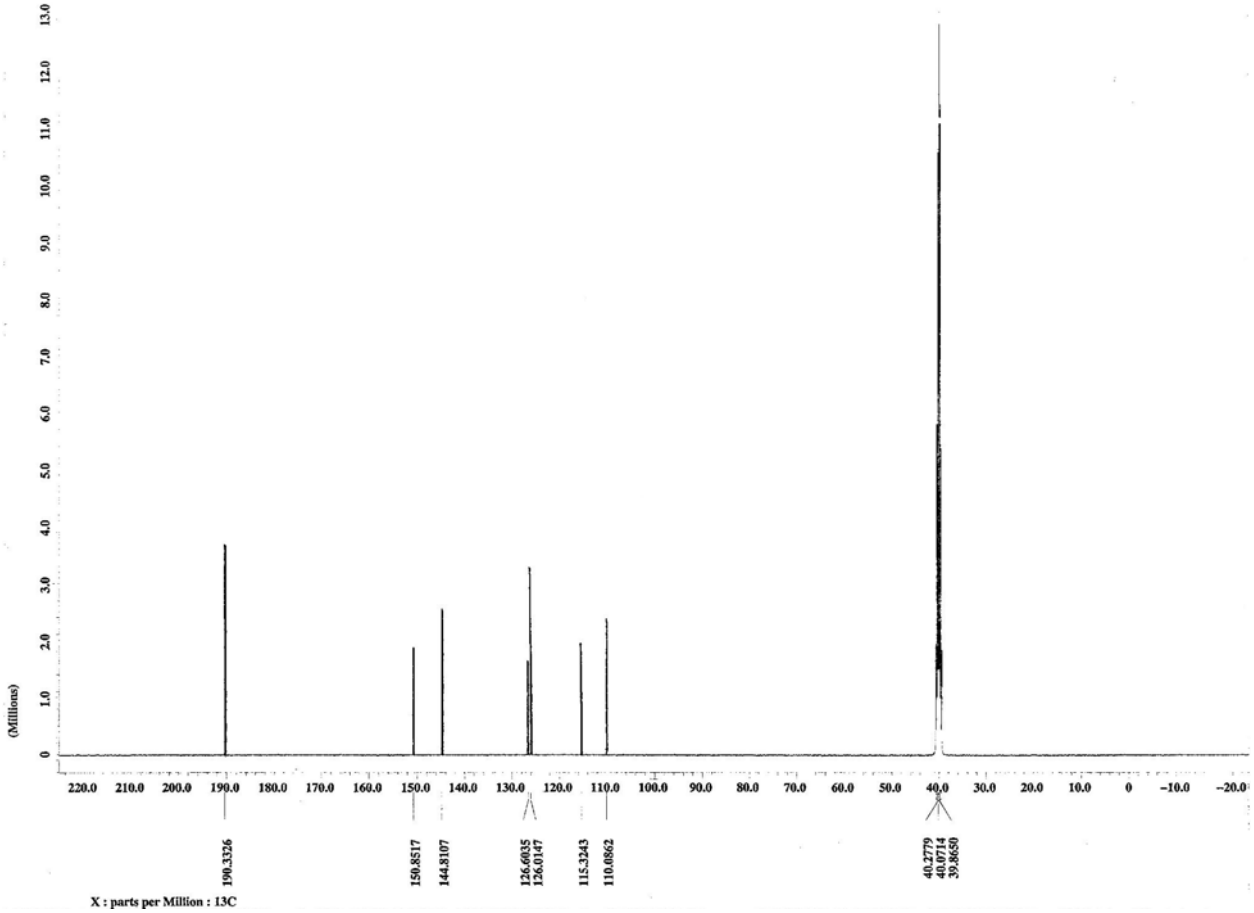
5



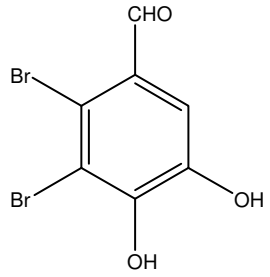
APPENDIX I. ^{13}C NMR Spectrum of Compound **5** in DMSO-d_6



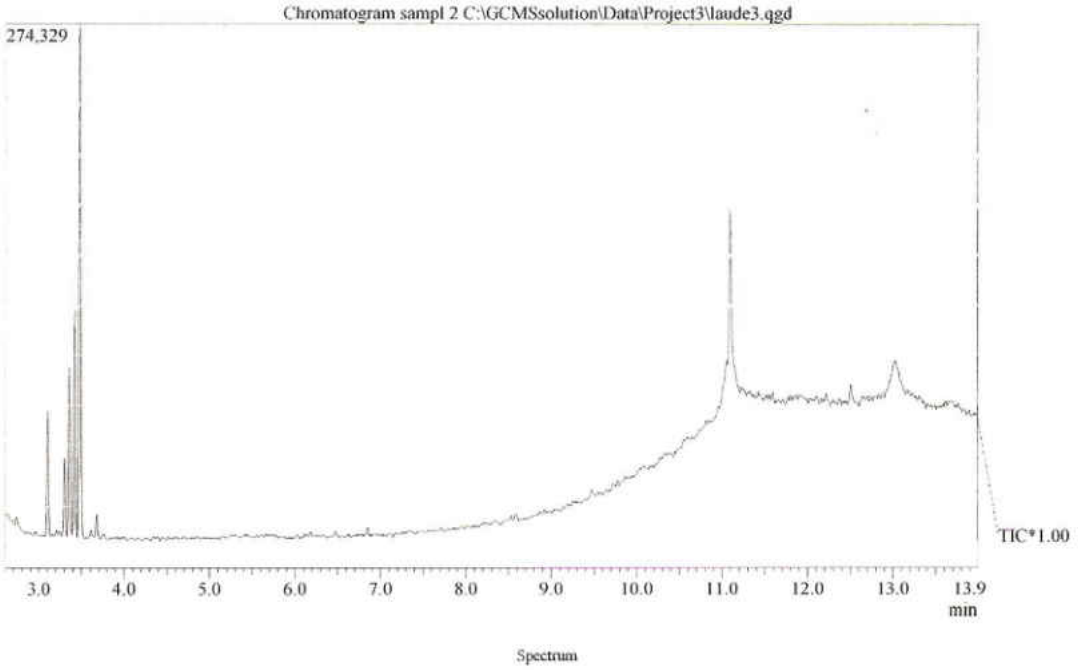
5

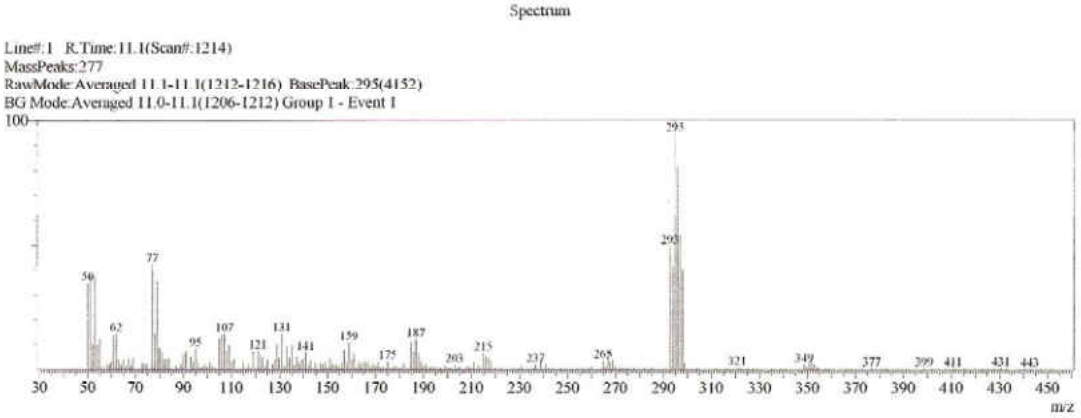


APPENDIX J. GCMS Spectrum of Compound **5** in acetone

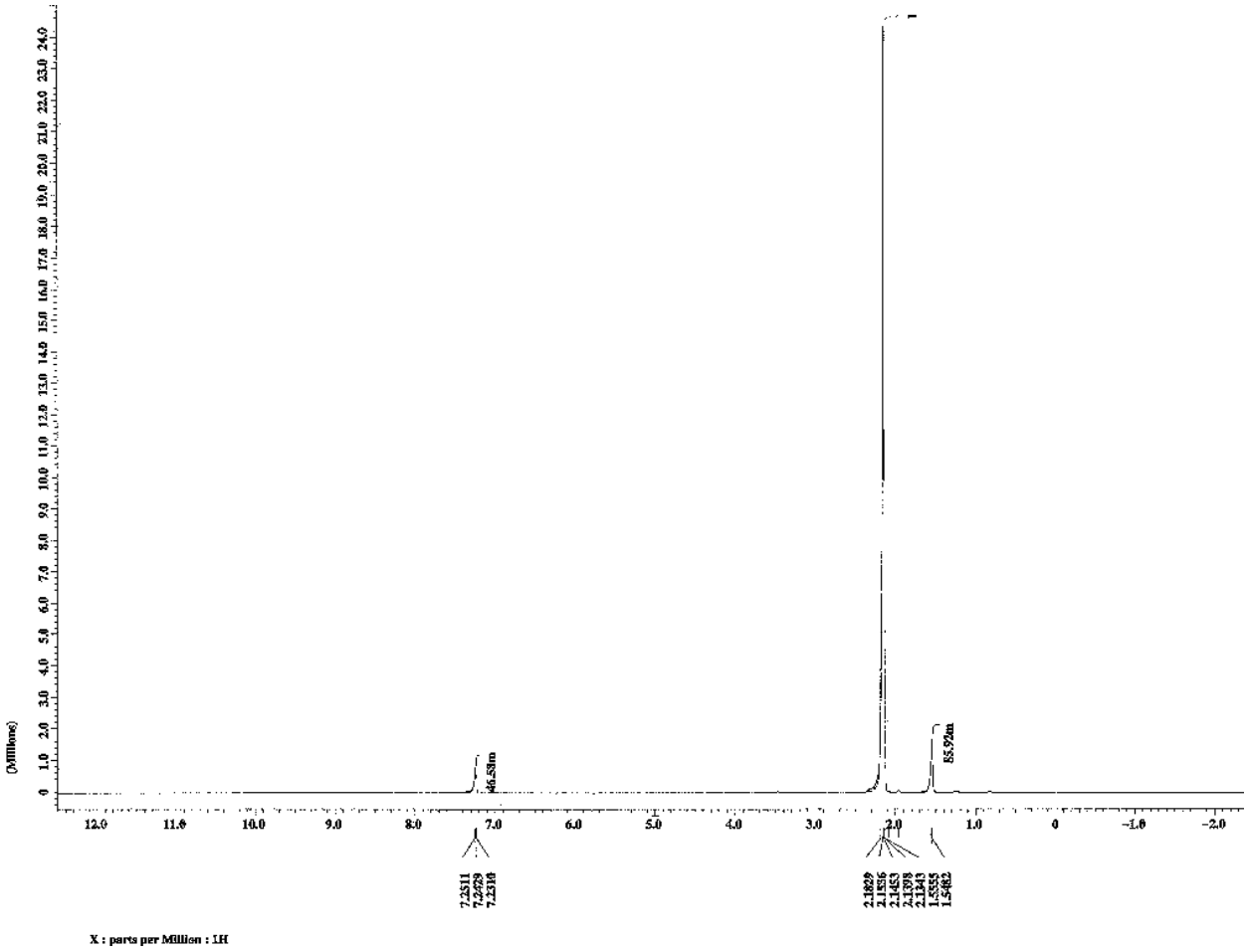


5

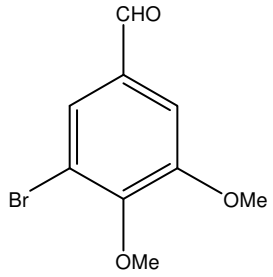




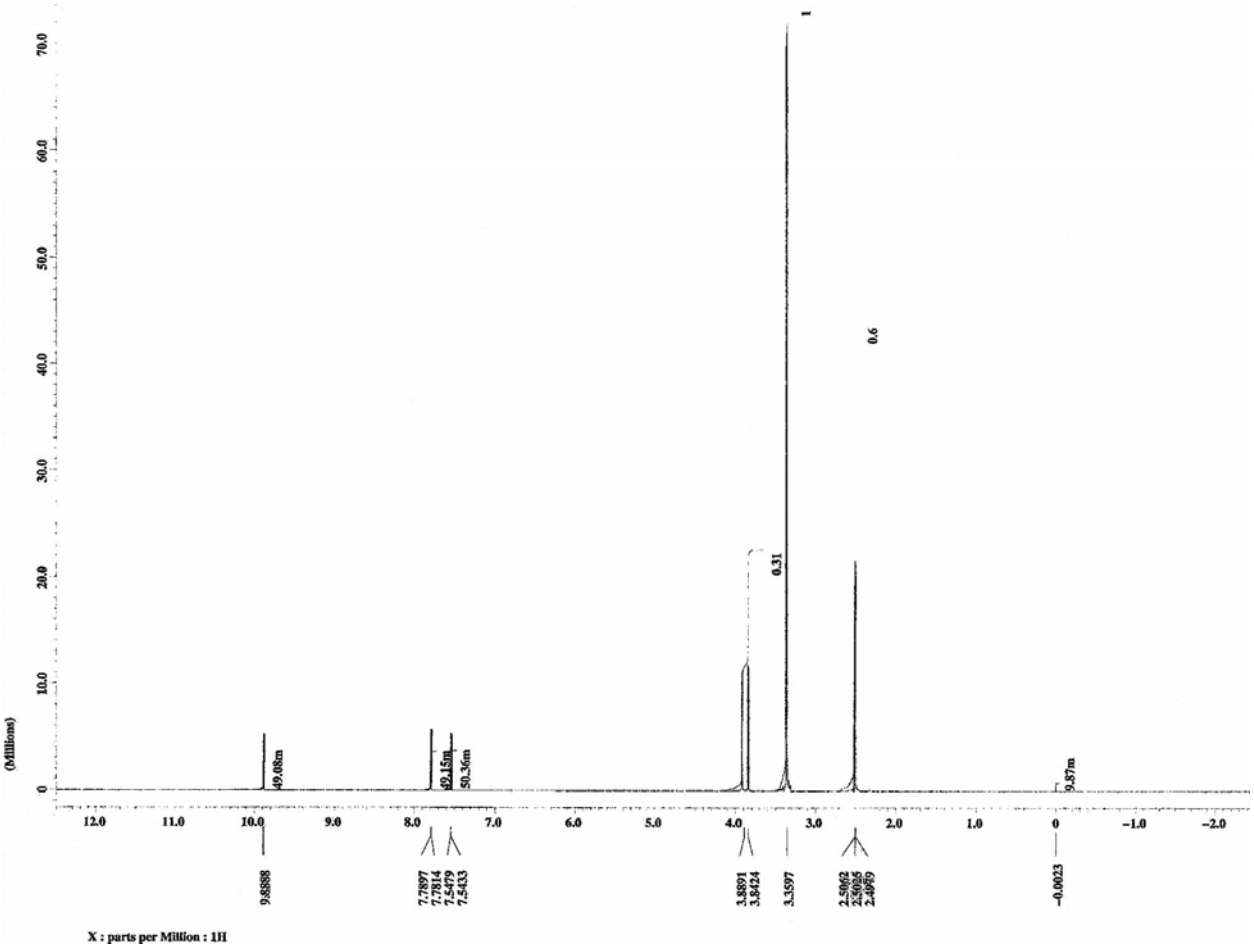
APPENDIX K. ^{1}H NMR Spectrum of MeI in $CDCl_3$



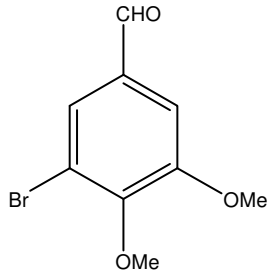
APPENDIX L. 1H NMR Spectrum of Compound **6** in $CDCl_3$



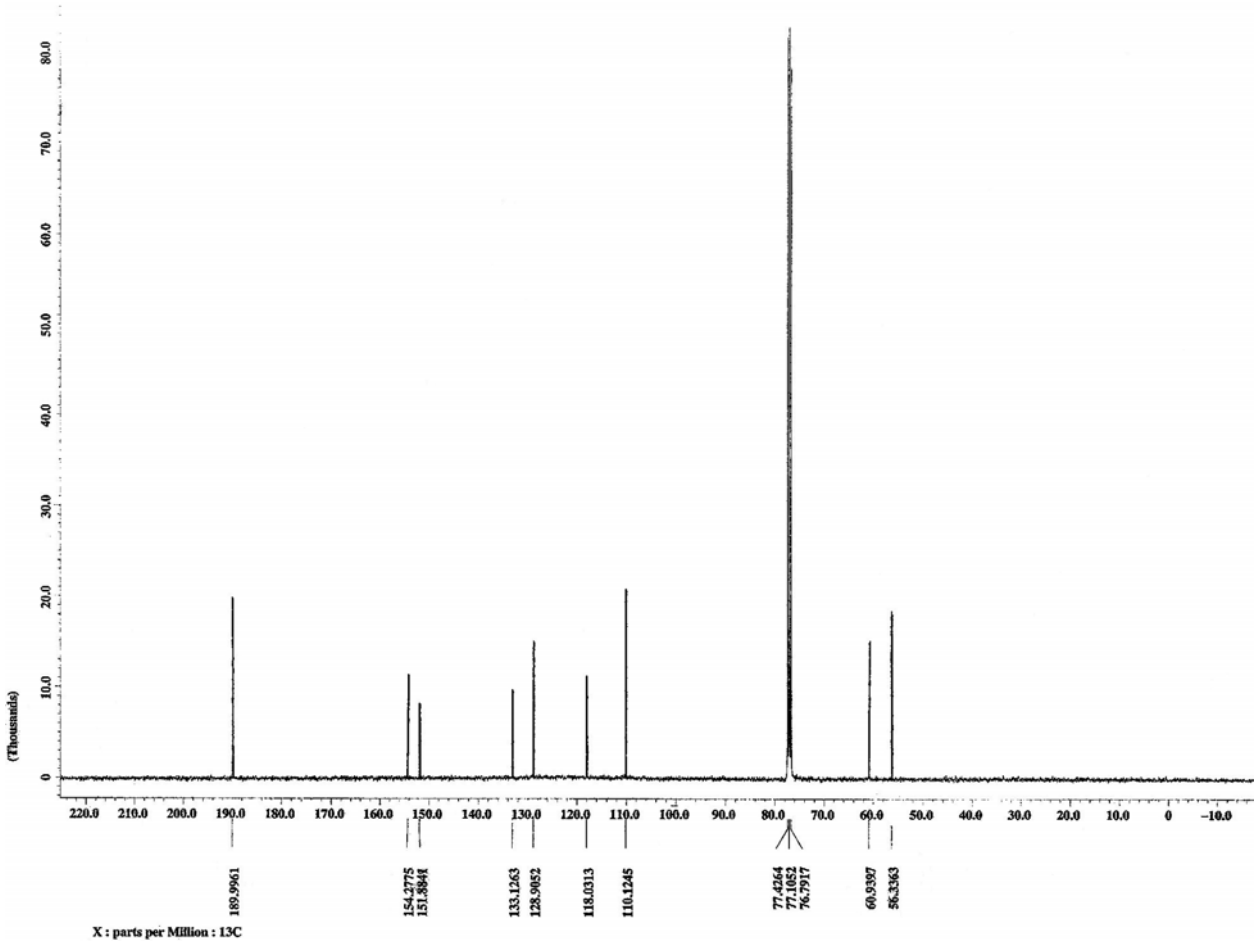
6



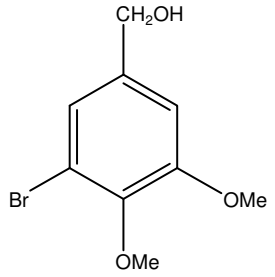
APPENDIX M. ^{13}C NMR Spectrum of Compound **6** in $CDCl_3$



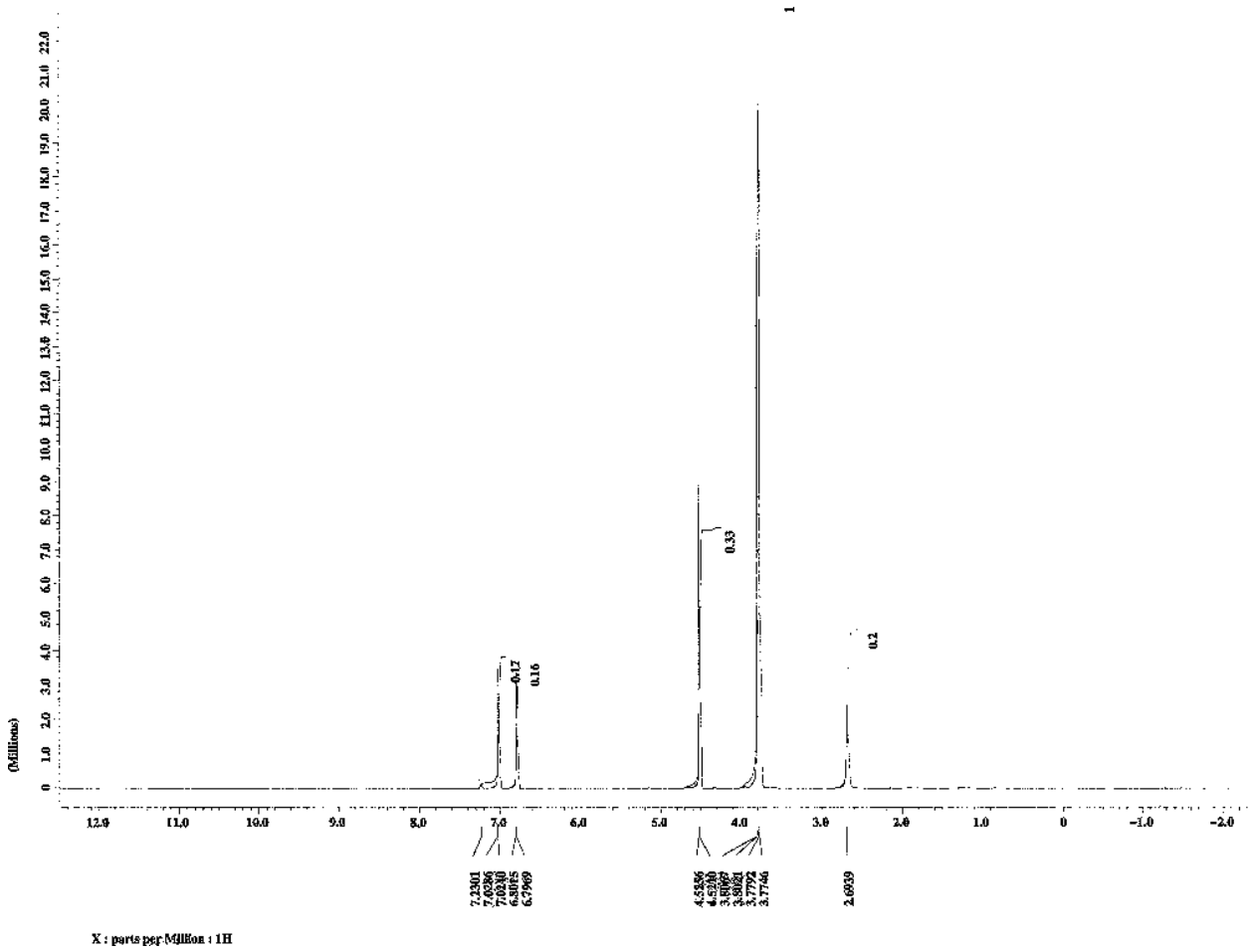
6



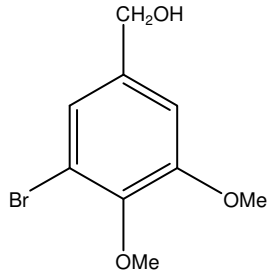
APPENDIX N. ^{1}H NMR Spectrum of Compound **7** in $CDCl_3$



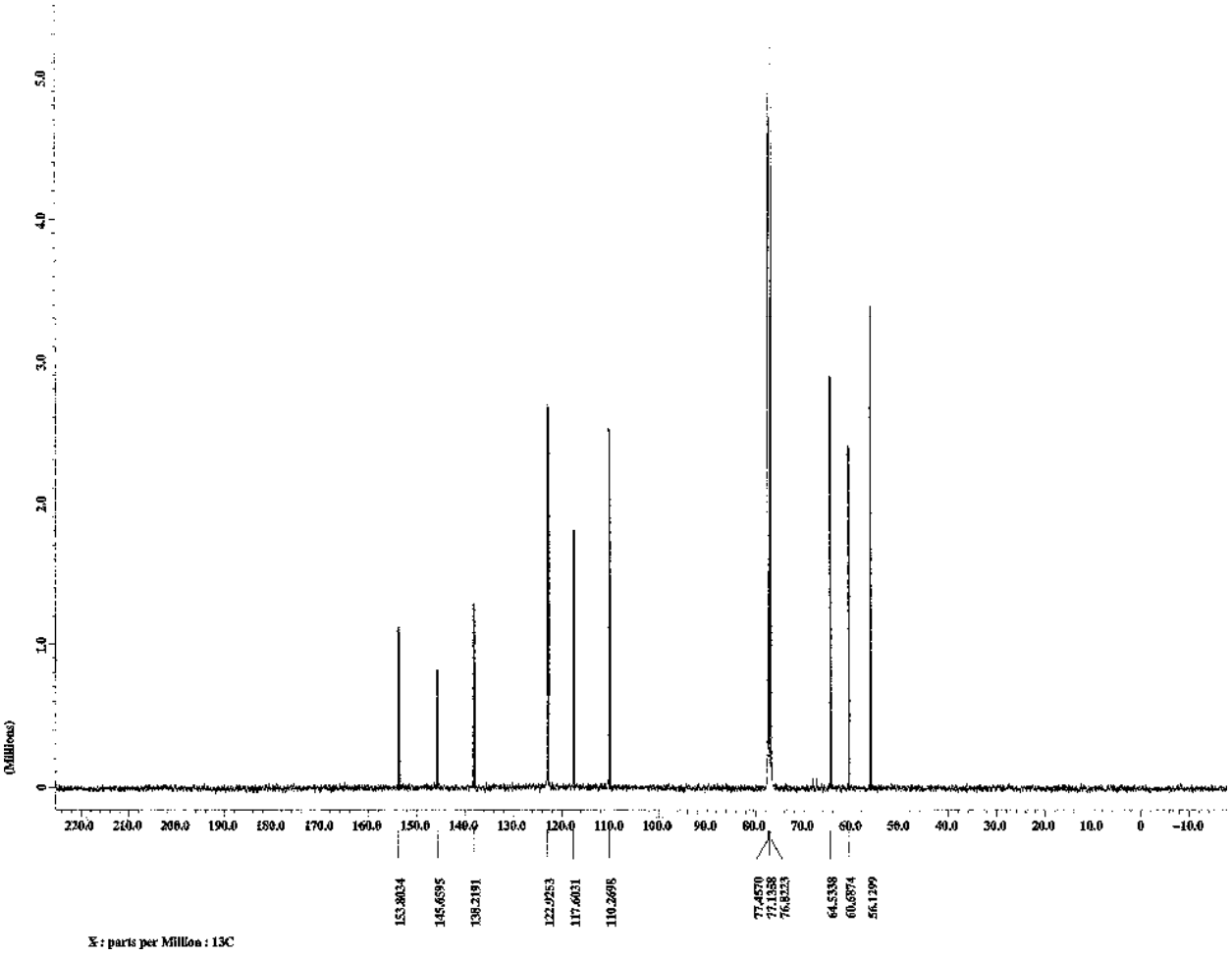
7



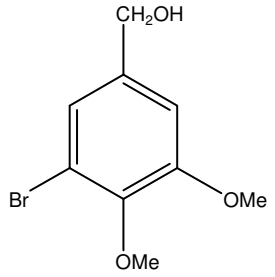
APPENDIX O. ^{13}C NMR Spectrum of Compound **7** in $CDCl_3$



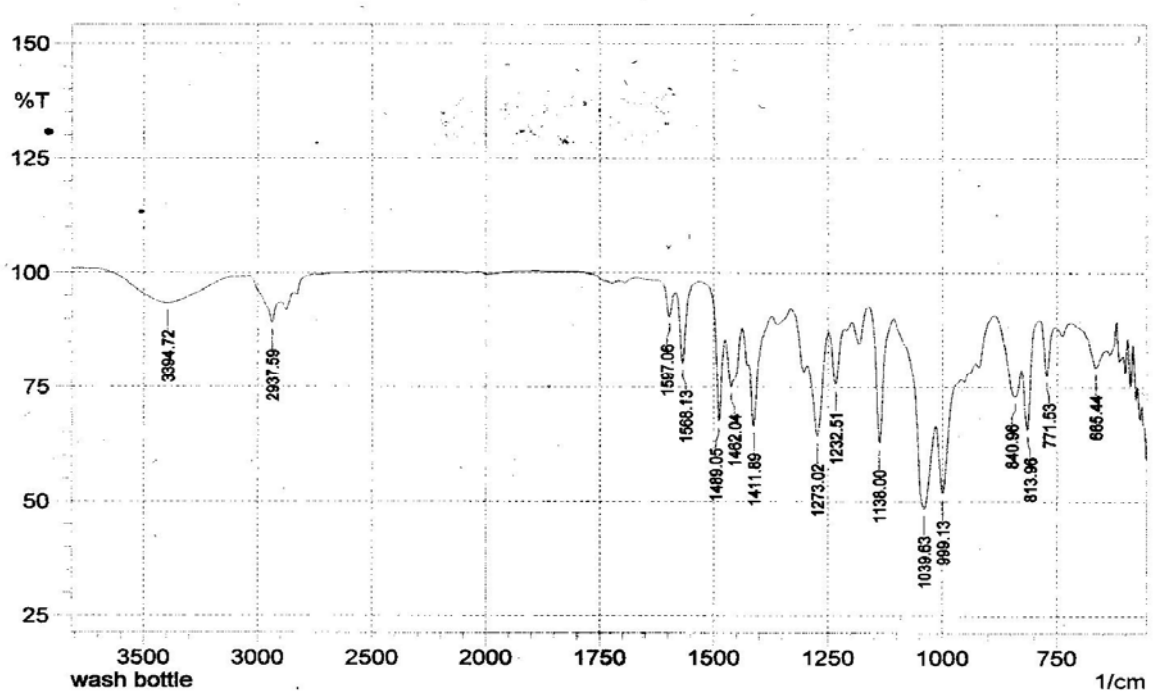
7



APPENDIX P. IR Spectrum of Compound **7**

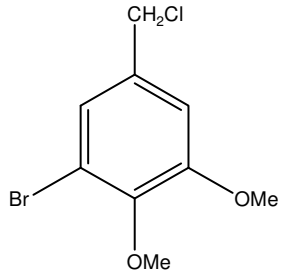


7

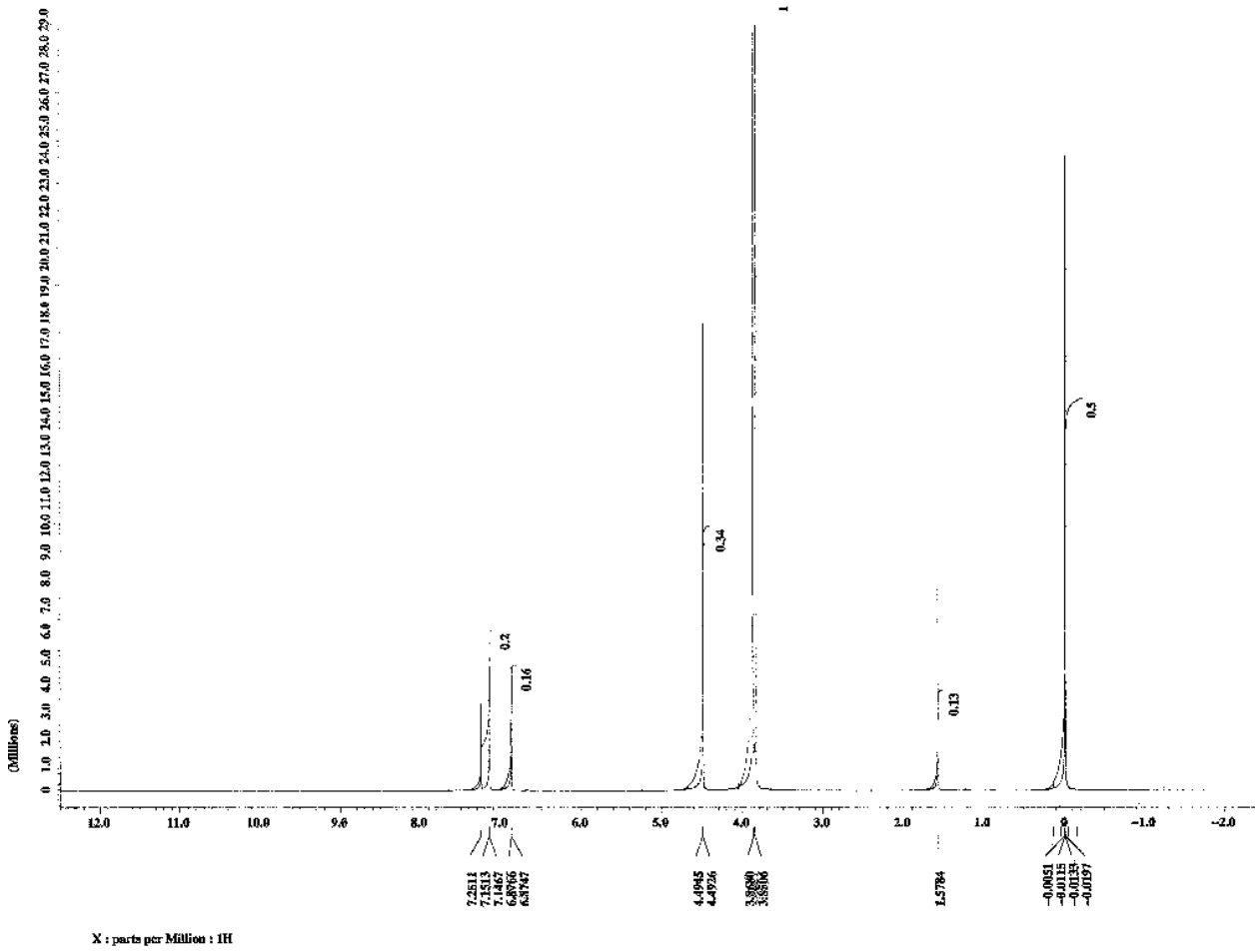


	Peak	Intensity	Corr. Intensity	Base (H)	Base (L)	Area	Corr. Area
1	445.56	13.97	70.22	453.27	443.63	3.47	3.07
2	665.44	79.22	5.81	719.45	642.3	5.59	0.62
3	771.53	77.45	11.62	788.89	754.17	2.5	0.76
4	813.96	65.63	17.03	827.46	788.89	3.99	1.22
5	840.96	72.97	7.94	885.33	829.39	5.3	1.13
6	999.13	51.89	17.54	1014.56	960.55	9.94	2.17
7	1039.63	48.51	24.01	1105.21	1016.49	14.35	4.43
8	1138	62.88	28.74	1159.22	1107.14	4.8	2.74
9	1232.51	75.55	11.86	1247.94	1213.23	2.94	0.91
10	1273.02	64.15	19.12	1294.24	1249.87	5.72	2.25
11	1411.89	66.52	16.07	1423.47	1373.32	4.62	1.2
12	1462.04	75.06	11.15	1473.62	1438.9	3.41	1.26
13	1489.05	67.57	21.24	1527.62	1475.54	2.94	1.2
14	1568.13	80.15	16.32	1583.56	1533.41	1.77	1.09
15	1597.06	90.41	6.2	1624.06	1583.56	0.84	0.31
16	2937.59	89.22	5.93	3037.89	2899.01	3.59	1.36
17	3394.72	93.41	0.14	3400.5	3107.32	4.98	0.3

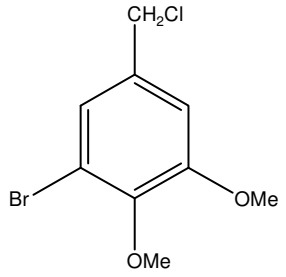
APPENDIX Q. ^{1}H NMR Spectrum of Compound **8** in $CDCl_3$



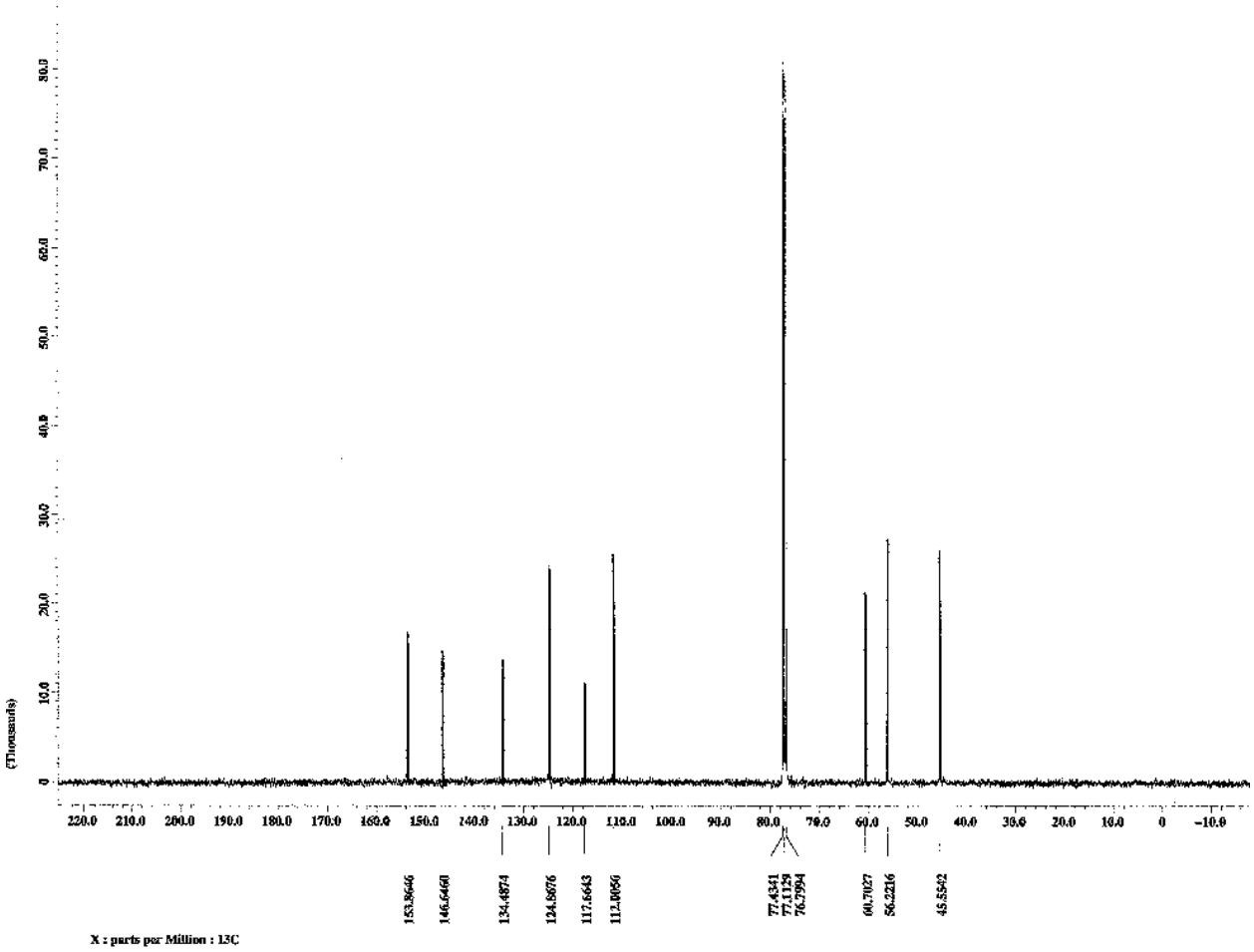
8



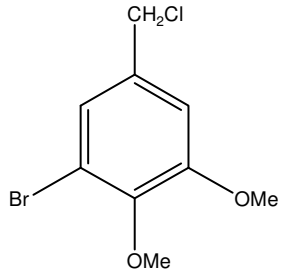
APPENDIX R. ^{13}C NMR Spectrum of Compound **8** in $CDCl_3$



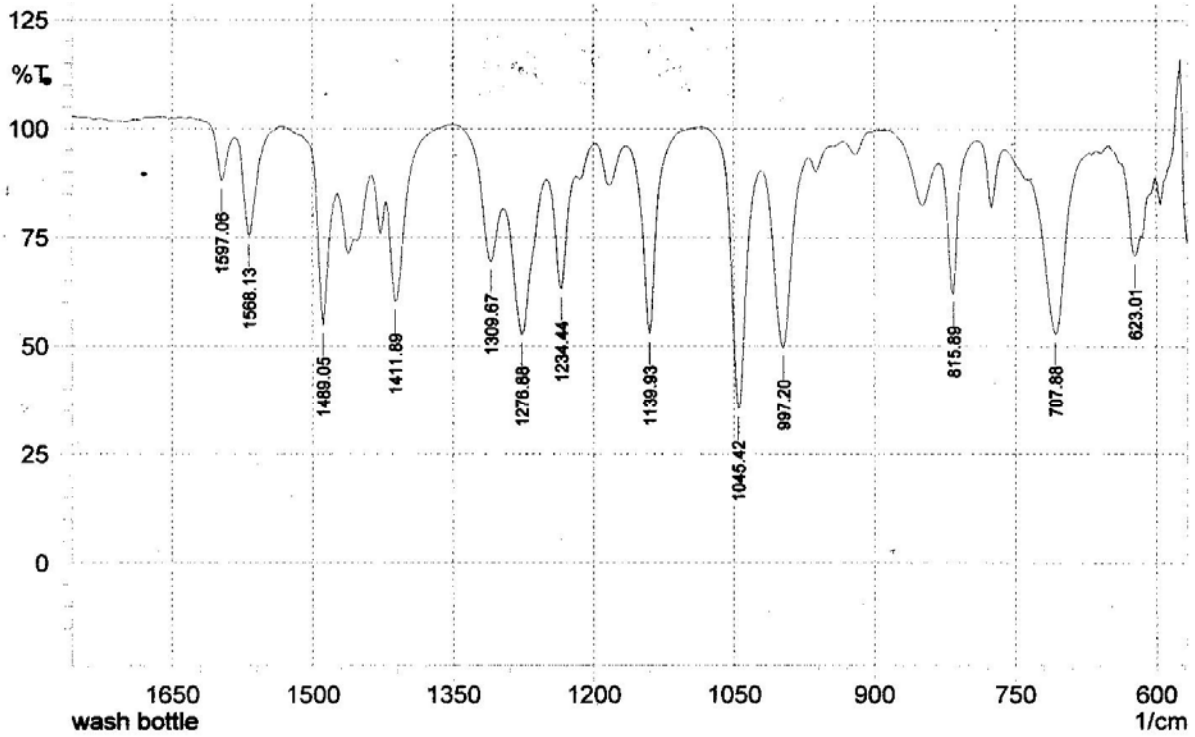
8



APPENDIX S. IR Spectrum of Compound **8**

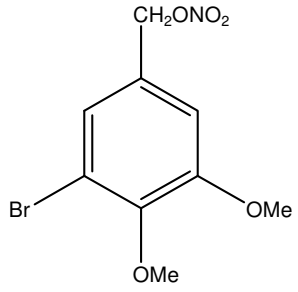


8

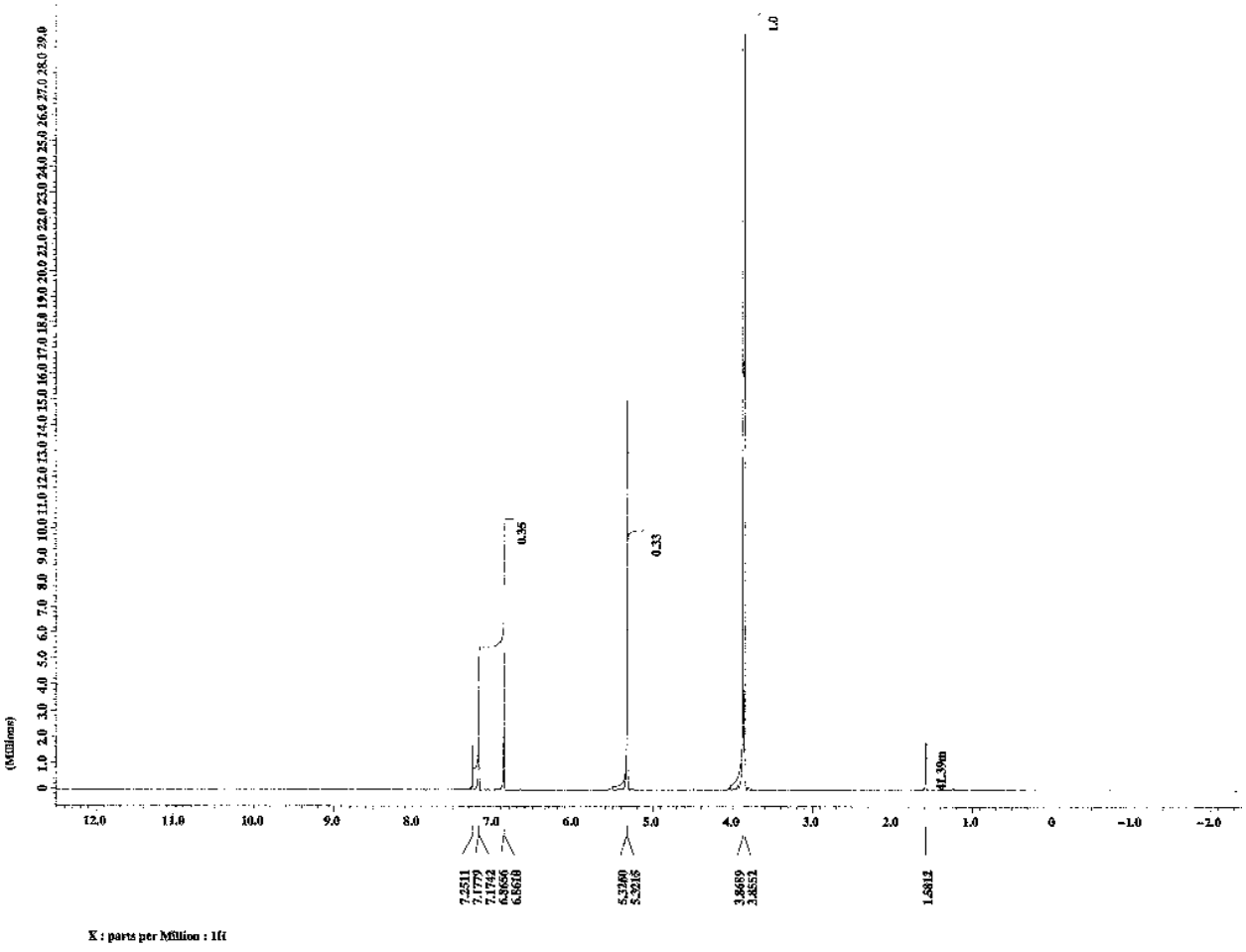


	Peak	Intensity	Corr. Intensity	Base (H)	Base (L)	Area	Corr. Area
1	623.01	70.86	8.32	650.01	617.22	2.37	0.1
2	707.88	52.86	41.81	759.95	673.16	8.6	6.56
3	815.89	62.04	32.17	831.32	790.81	2.9	1.95
4	997.2	49.62	42.2	1020.34	970.19	6.79	4.96
5	1045.42	35.42	58.65	1083.99	1022.27	7.36	6.04
6	1139.93	52.81	44.64	1165	1085.92	4.43	3.85
7	1234.44	63.09	25.6	1249.87	1219.01	3.37	1.76
8	1276.88	52.67	31.75	1296.16	1251.8	7.01	3.86
9	1309.67	69.33	16.36	1350.17	1298.09	3.16	1.18
10	1411.89	60.25	25.55	1421.54	1352.1	3.85	1.93
11	1489.05	54.84	33.56	1529.55	1475.54	3.69	2.03
12	1568.13	75.28	23.47	1583.56	1535.34	1.89	1.72
13	1597.06	88.01	11.22	1631.78	1583.56	0.45	0.49

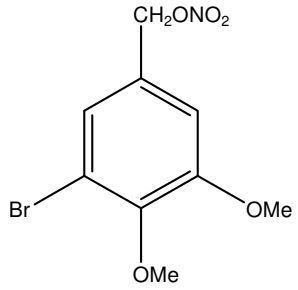
APPENDIX T. ^{1}H NMR Spectrum of Compound **9** in $CDCl_3$



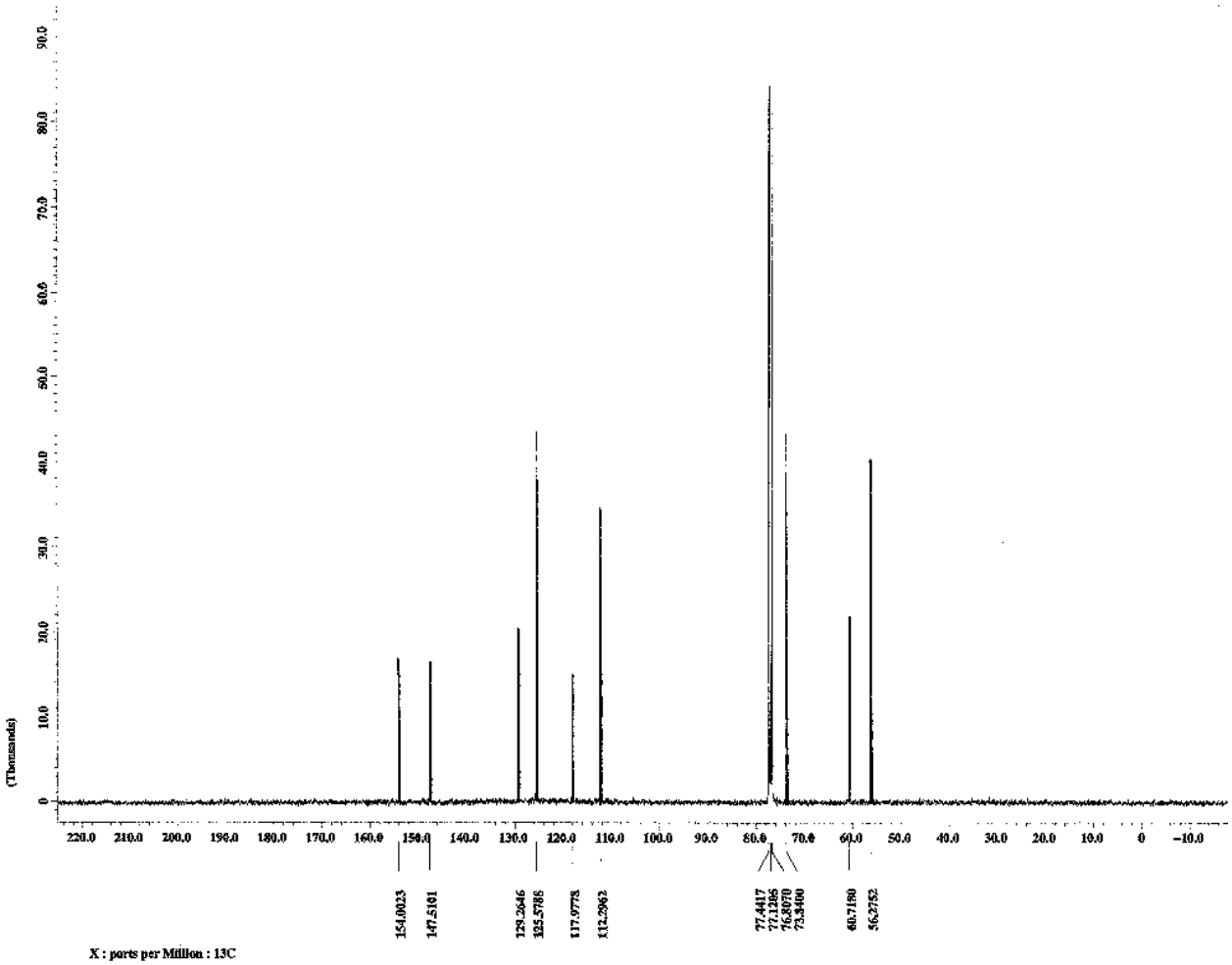
9



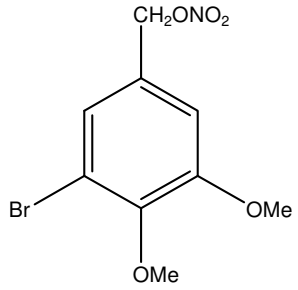
APPENDIX U. ^{13}C NMR Spectrum of Compound **9** in $CDCl_3$



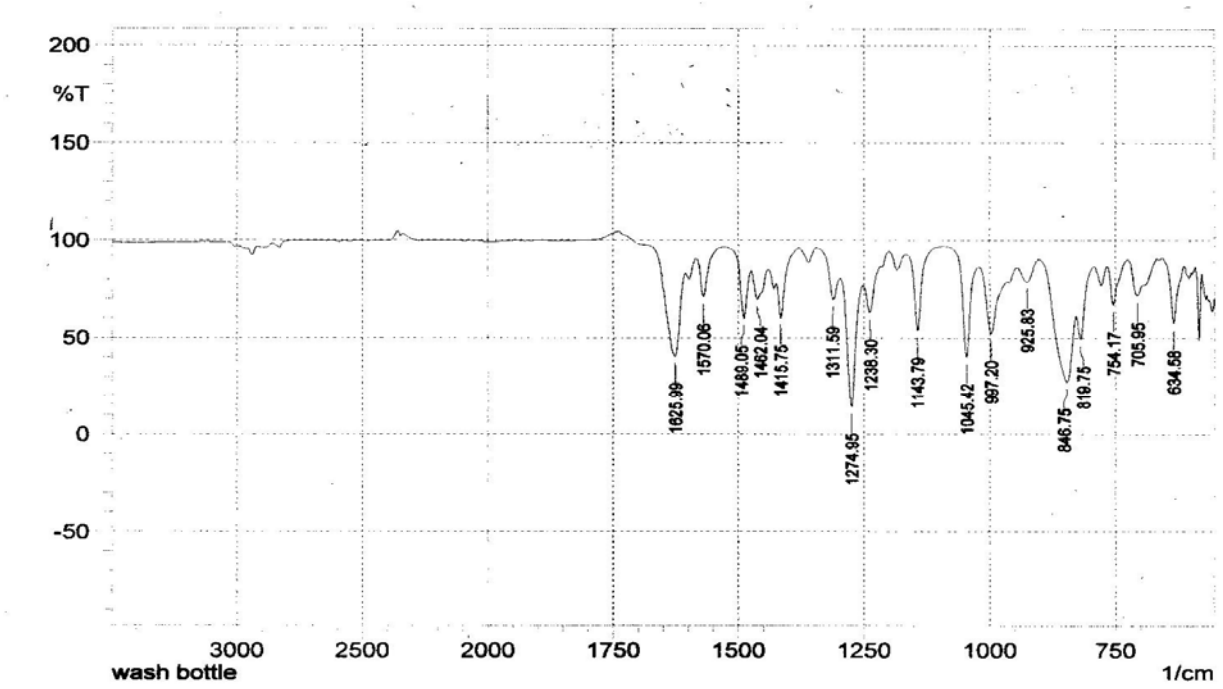
9



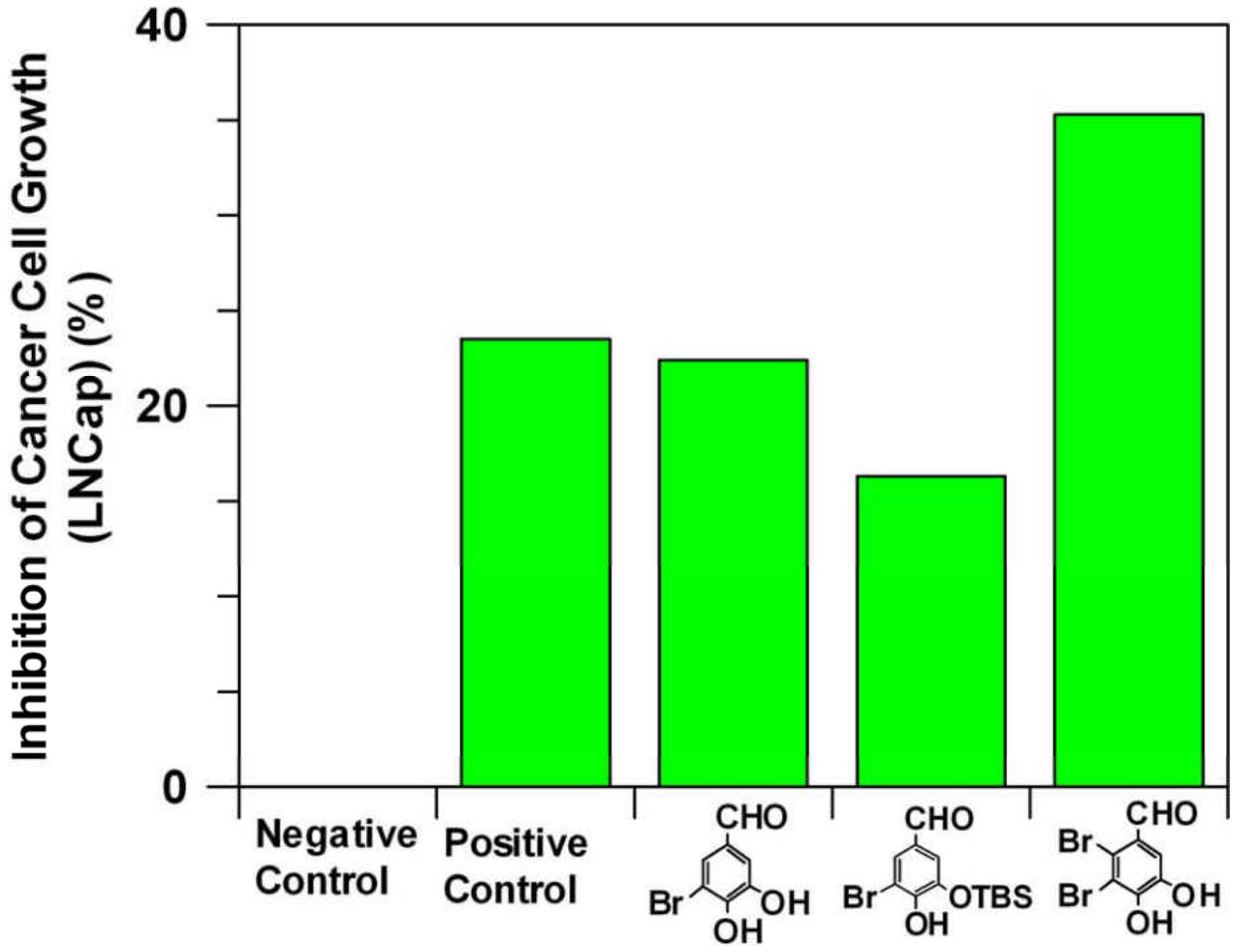
APPENDIX V. IR Spectrum of Compound **9**



9



	Peak	Intensity	Corr. Intensity	Base (H)	Base (L)	Area	Corr. Area
1	634.58	57.58	31.03	655.8	615.29	4.75	2.62
2	705.95	71.8	9.74	725.23	696.3	3.08	0.76
3	754.17	67.03	20.75	765.74	727.16	3.76	1.72
4	819.75	49.39	17.38	827.46	792.74	5.65	1.26
5	846.75	27.24	41.08	900.76	829.39	20.72	11.46
6	925.83	78.84	9.1	945.12	902.69	3.3	0.99
7	997.2	52.14	30.97	1020.34	966.34	8.68	4.12
8	1045.42	40.31	49.6	1089.78	1022.27	7.35	4.78
9	1143.79	53.79	40.28	1165	1091.71	5.15	3.51
10	1238.3	62.97	17.23	1249.87	1217.08	4.25	1.39
11	1274.95	14.17	64.46	1296.16	1251.8	14.87	10.2
12	1311.59	69.63	15.25	1344.38	1298.09	3.52	1.03
13	1415.75	59.97	21.2	1423.47	1381.03	3.74	1.29
14	1462.04	69.56	15.07	1473.62	1440.83	3.88	1.52
15	1489.05	59.84	27.61	1525.69	1475.54	4.14	1.96
16	1570.06	70.83	21.24	1583.56	1529.55	3.17	1.64
17	1625.99	40.55	45.38	1737.86	1606.7	12.04	8.26


Inhibition of Cancer Cell Growth (LNCap) (%)
40
20
0
Negative Control
Positive Control
CHO
Br
OH
OH
CHO
Br
OTBS
OH
CHO
Br
Br
OH
OH

VITA

LAUDE BANNERMAN-AKWEI

Personal Data	Date of Birth: January 6th 1979 Place of Birth: Accra, Ghana Marital Status: Single
Education	Bsc Chemistry, University of Cape Coast Ghana 2004 MS Chemistry, East Tennessee State University, Johnson City, Tennessee. 2008
Professional Experience	Teaching Assistant; University of Cape Coast, Ghana. 2004-2005 Graduate/Teaching Assistant, East Tennessee State University, College of Arts and Sciences, 2006-2008
Publication	Determination of esterase activity of SABP2 using fluorescence spectroscopy ACS Abstract - 2007